Comprehensive extraction and NMR-based Metabolomics : novel approaches to natural products lead finding in drug discovery

Yuliana, N.D.

Chapter 1

Herbal medicine for obesity treatment: a review

Nancy Dewi Yuliana [1,2], Muhammad Jahangir[1], Henrie Korthout[3], Young Hae Choi[1], Hye Kyong Kim[1], Robert Verpoorte[1]

[1]Div. Pharmacognosy, Section Metabolomics, Institut of Biology, Leiden University, Einsteinweg 55, 2333 CC Leiden, The Netherlands.
[2]Dept. Food Science and Technology, Bogor Agricultural University, IPB Darmaga Campus, Bogor 16680, Indonesia.
[3]Fytagoras BV Plant Science, Sylviusweg 72, 2333 BE Leiden, The Netherlands.

Abstract

The obesity drug development is presently not a bright and successful story. So far, drugs reported to be effective, either from synthetic or natural sources, mostly stimulated controversy due to serious adverse effects, which ended with stopping clinical trials or even withdrawal from the market. However, obesity and its co-morbidity have become rapidly a major problem in both developed and developing countries. This has encouraged pharmaceutical companies and academia to keep on struggling on developing novel effective but safe obesity drugs, and on characterizing novel obesity drug targets. From existing scientific work on obesity drug discovery and commercial slimming preparations, compounds originating from nature, especially from plants, seem to be the first choice. Traditional belief that herbal medicine is safer than synthetic ones is one of the classical arguments, although scientifically this is not always true (ban on *Ephedra*, for example).

But in general, it has been widely acknowledged that plant compounds, with their unique scaffolds and rich diversity are an unlimited source of novel lead compounds. All work summarized in this review is focused on screening plant materials by targeting various important pathways related to energy homeostasis, either by *in-vivo* or *in-vitro* experiments. So far only a few of them have come quite far in the development track, leading to some patented products (e.g. *Hoodia sp.*). The mechanisms of action of these preparations have been elucidated until gene

transcription level; but reports on efficacy and safety in humans are still mostly inadequate.

Keywords: Obesity, drugs, plants, energy homeostasis, natural products

1.Accepted in Obesity Reviews: Yuliana *et al*., 2010. Comprehensive review on herbal medicine for energy intake suppression.

2.Accepted in Critical Reviews in Food Science and Nutrition. Yuliana *et al*., 2011. Plant-derived food ingredients for stimulation of energy expenditure.

3. Submitted to Obesity Facts. Yuliana *et al*., 2011. A review on herbals with adipogenesis inhibition activity.

Introduction

Obesity has become a serious global health problem both in developed and developing countries. Obesity is not only a cosmetic problem but it associates with several diseases, especially cardiovascular diseases, type 2 diabetes, degenerative joint diseases, and cancer, therefore diminishing life expectancy and lowering the quality of life of affected individuals (16-19). Obesity is also an extremely costly health problem which accounts for 2-6% of total health care costs in developed countries (20). According to WHO standards, a body-mass index (BMI) of 25.0 kg/m^2 or higher is categorized as overweight; the BMI 30.0 kg/m^2 or more as obese. Obesity occurrence relates to the regulation of energy intake, energy expenditure, and energy storage in the body. The significant contribution of genetic factors to human obesity involving susceptible genes and the respective pathways of energy expenditure and food intake has been reviewed by several authors (21-25). Although it is well-understood that a positive energy balance results in gaining weight is the start of obesity, it is also influenced by other factors such as behavior, age, and environment (20, 26). To reverse this epidemic, the long term efficacy of dietary and behavior counseling have not met the expectations yet, the pharmacotherapeutic intervention thus becomes an alternative although there is a necessity for appropriate prescription of anti-obesity drugs (27).

It has been suggested that pharmacotherapeutic intervention is only recommended under specific conditions (28), where the patient condition prompts to disease development and the safety profile of drugs is acceptable. WHO recommends drugs intervention for patient with BMI above 30 kg/m^2, or BMI more than 27 kg/m^2 when additional co-morbid factors are present (17, 29). Surgical treatment is another option which is able to give long term efficacy compared to conventional methods (30). But its use is restricted to more severe obese patient with BMI more than 40 kg/m^2 or BMI more than 35 kg/m^2 with the presence of more severe co-morbid factors (16).

Historically there is almost no success in anti-obesity drug development due to the low efficiency and undesired side effects (31). Some disappointing cases were also reviewed for agents that were originally tried for more than 70 years such as thyroid extract, dinitrophenol, amphetamine, some norepinephrine reuptake inhibitors, serotonergic agents, and fenfluramine/phentermine (32). Currently only Orlistat

(Xenical®, Hoffman-La Roche), an inhibitor of pancreatic and gastrointestinal lipases which is able to prevent the absorption of approximately 30% of dietary fat (17), has been approved by Food and Drug Administration (1) in the United States of America, the Therapeutic Products Directorate (TPD) in Canada, and the European Medicines Agency (EMEA) for long-term use in obesity. The use of Rimonabant (Acomplia, 20 mg, Sanofi-Aventis, Paris, France), an appetite suppressant acting as cannabinoid (CB1) receptor antagonist previously approved in European Union since June 2006, has been recommended to be suspended by EMEA in October 2008, as can be read on their website http://www.emea.europa.eu. The decision was taken after an extensive review on its safety which mentioned that the risk of psychiatric side effects, including depression, sleep disorders, anxiety and aggression, was doubled in patients taking Acomplia, compared to patients taking placebo. It was reported that of 36.000 patients taking Acomplia, 5 suicide cases occurred, compared to one case in patients taking placebo. Apparently the CB1 receptor is a difficult target for treating obesity. Surprisingly, in January 2010 EMEA also recommended to suspense the use of another appetite suppressant, Sibutramine (Meridia®, Reductil®, Raductil®, Ectiva®, Abbott Laboratories, Illinois, USA), a monoamine reuptake inhibitor. The data from Sibutramine Cardiovascular Outcome Trial showed that there is an increased risk of serious, non-fatal cardiovascular events (e.g. stroke, heart attack) in patient taking Sibutramine compared with placebo, prolonging the list of the dissatisfying stories of obesity drug development.

Nature is the most productive source of leads for novel drugs against various pharmacological targets including cancer, HIV/AIDS, Alzheimer's, malaria, and pain (33, 34). Of a number of reviews describing the use of dietary supplements for weight loss management, agents from natural sources are predominant, although the efficacy is stated as not convincing (35-41). Few of them specifically deal with the potential of herbal medicine (35, 39, 41). The mechanisms of action of medicinal plants on obesity can be divided as direct and indirect action (35). Medicinal plants with direct action combat obesity by stimulating the rate of metabolism and suppressing the appetite. Synephrine, xanthine, and caffeine are active principals found in several medicinal plants that stimulate metabolism. Plants may suppress appetite by their high dietary fiber content, but the effect will only be achieved at high dose use. Indirect mechanisms

which might be useful to treat obesity are diuretics and central nervous system suppressants, though the first type only affects the weight by lowering the body water content (35).

As obesity results from the imbalance between energy intake and energy expenditure several strategies can be applied for obesity drug development; reduction of energy intake by appetite suppression; inhibition of nutrient absorption; increase of energy expenditure; and modulation of fat (6). The ideal obesity drugs must affect the homeostasis of body fat storage which may be reached by a combination of drugs (42), although combination of drugs therapy plus dietary restriction, exercise and counseling might be more effective (43).

In this chapter, plants reported for anti-obesity activity are reviewed and grouped based on the possible mechanisms involved. The efficacy and safety are also discussed.

1. Energy intake reduction

A. Appetite regulation

Food restriction is the first line treatment of obesity (44). A small increase of calories as 20 – 30 kcal per day within several years may raise the body weight significantly which will lead to obesity. The phrase "If human beings are the most intelligent life force on this planet, why is it that they cannot adjust their (eating) behavior by the very small amounts which would be required for weight stability rather than weight escalation?" (45) underlines that appetite control is crucial for long term regulation of body weight.

The complexity of appetite regulation has been reviewed by several authors (43-51). Approximately 40 orexigenic and anorexigenic hormones, neuropeptides, enzymes, other cell signaling molecules and their receptors are involved in a complex human appetite and satiety regulation (43). However, leptin and insulin are the most important signals, others such as leptin/insulin mediators affect the central nervous system (47). These abundant signaling molecules are positively or negatively correlated with each other by mechanisms which are still not fully understood.

The hunger and satiety signaling molecules are produced centrally in the brain and peripherally in e.g. the digestive tract, adipose tissue and liver (47, 52). The most important part of the brain responsible for appetite regulation is the hypothalamus arcuate nucleus (ARC). An illustration of parts of the brain, neurons, and peripherally secreted hormones which are important for appetite regulation and their interaction is presented in Figure 1. The ARC and brainstem neurons receive and translate information from peripheral hormones about acute nutritional status and adiposity level, while neural and endocrine signaling from gastrointestinal tract (GI) regulate appetite on the short term (53). The ARC contains the orexigenic neurons neuropeptide Y (NPY) and agouti-related protein (AgRP) (54); and anorexigenic neuron pro-opiomelanocortin (POMC) - cocaine-amphetamine-regulated transcript (CART) (55). Leptin and insulin regulate both types of ARC neurons by inhibiting NPY and stimulating POMC (56), beside this, NPY/AgRP – POMC/CART inhibitory cross-talk also exists (54, 57). The other orexigenics, orexin A and B, are expressed in the lateral hypothalamic area (LHA). Both are inhibited by POMC/CART and stimulated by NPY (47).

The peripheral signals enter the ARC via the brainstem area, the nucleus tractus solitarus (NTS) (49, 52). These peripheral signals encode information about acute nutritional state and adiposity. Leptin transduces the size of adipose tissue to the brain. However, targeting on this pathway is not a choice since the obese mostly suffers from leptin resistance. Short term appetite regulation by signals from gastrointestinal tract reflects the postprandial satiety and hunger felt before a meal, and might be a more reasonable target for obesity treatment (53). The gastrointestinal tract is considered as the largest endocrine organ in the body which secretes more than 20 distinct hormonal regulatory peptides, mostly sensitive to nutritional status of the gut, thus mediating a short term appetite regulation (53). The most well-known GI peptides important for appetite regulation has been reviewed (50). Cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), and peptide YY (3-36) [PYY(3-36)] seem to be the only ideal models of anorexigenic signals produced in the GI, since other hormones do not show any responses in knock-out animals or antagonist activity (52). Ghrelin is the only orexigenic GI peptide hormone known (58). It was suggested that ghrelin opposes leptin action in NPY/AgRP (59), while the CCK anorexigenic effect is probably enhanced by leptin/insulin (60).

The signals from both pathways are affecting the second level of the hunger-satiety neuronal signaling area; paraventricular nucleus (PVN), perifornical area (PFA), and LHA (47, 61) to give an orexigenic or anorexic response, depending on which pathway is activated (47, 62).

Prolonging consumption of palatable food (fat and sugar rich diet) may alter the regulation of abovementioned appetite regulating peptide expression, as has been reviewed elsewhere (62). With palatable food, registration of the attractive taste of the food activates the reward system and interferes with hypothalamic appetite regulation. Satiety signals expression are increased but the satiety response to circulating leptin, insulin, and cholecystokinin is blunted, while hunger signals are either increased (e.g. AgRP and NPY), or decreased (e.g. ghrelin). Palatable food might also induce resistance to some satiety signals such as leptin, insulin, and CCK, leading to overeating. There are three neurotransmitter systems important for food rewarding response, all are located between the nucleus accumbens and lateral hypothalamus: the opioid, dopamine, and serotonin system (62). In a recent review, the cannabinoid system was hypothized to also influence the feeding behavior via this reward circuitry (63). Synergism between the cannabinoid and the opioid system has been reported as well (64).

The existence of fuel sensing in CNS has been reviewed (65). Some specific neurons in ventromedial (VMN), ARC, and NTS have been found to be sensitive to a very narrow fluctuation of glucose level in CNS. A specific neuron subset gives positive feedback while the other gives negative feedback to an increase of glucose level, resulting in food intake reduction (66). Glucose, lipids and fatty acids sensing exist not only in the hypothalamus, but also particularly in the melanocortin system (67). Examples are the reduction of food intake after the administration of fatty acid synthase (68), carnitine palmitoyltransferase-1 (CPT-1), and oleic acid (67, 69). Reduction in hypothalamic adenosine monophosphate–activated protein kinase (AMPK) activity is also found to decrease food intake (70).

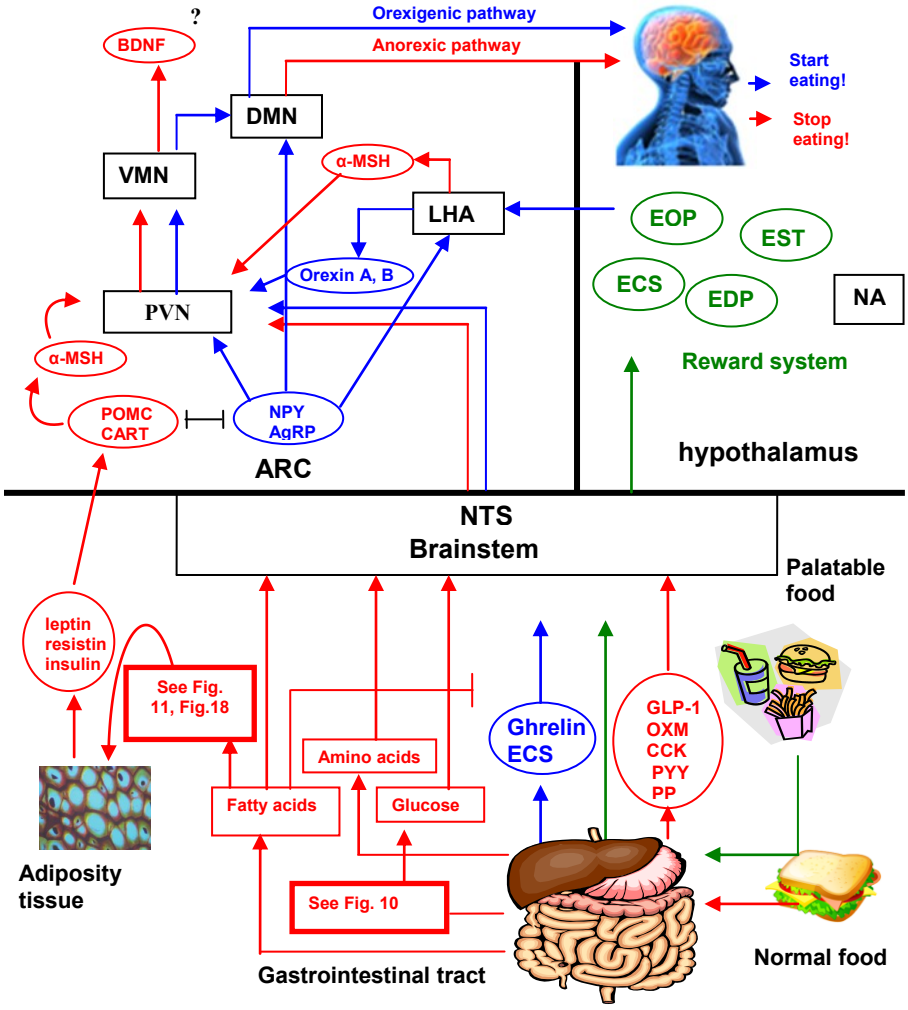


Figure 1. Central and peripheral appetite regulation. Area of appetite regulation in the brain: NTS (Nucleus tractus solitarus), ARC (Arcuate nucleus), NA (Nucleus accumbens), PVN (Paraventricular nucleus), LHA (Lateral hypothalamic area), VMN (Ventromedial nucleus), DMN (Dorsomedia nucleus), Orexigenic neuropeptides: NPY (Neuropeptide Y), AgRP (Agouti-related protein), Orexin A and B. Anorexigenic neuropetides: POMC (Pro-opiomelanocortin), CART (Cocaine- and amphetamine-regulated transcript), α-MSH (α-melanocyte-stimulating hormone), BDNF (Brain-derived neurothropic factor, detail pathway to be determined). Peptides involved in reward systems: ECS (Endocannabinoids), EOP (endogenous opioids), EST (Endogenous serotonin), EDP (Endogenous dopamine). Peripheral orexigenics from gastrointestinal tract: Ghrelin, ECS (Endocannabinoids). Peripheral anorexigenics: GLP-1 (Glucagon-like peptide 1), OXM (oxyntomodulin), CCK (Cholecystokinin), PYY (Peptide YY), PP (Pancreatic polypeptide). Hormones signaling an adiposity size: leptin, resistin, insulin. Red arrow: anorexigenic pathway; Blue arrow: orexigenic pathway; Green arrow: reward system. ——| = inhibit, |—— = inhibitory crosstalk.

The mammalian target of Rapamycin (mTOR) protein, a serine-threonine kinase, which regulates cell-cycle progression and growth, was found to be expressed in 90% of ARC NPY/AgRP neurons and in 45% of ARC POMC/CART. Centrally administered L-leucine increase mTOR expression followed by food intake and body weight reduction. Leptin also modulates hypothalamic mTOR signaling, and leptin's effect on food intake is mTOR-dependent (71).

Botanicals with appetite suppressant activity:

1. *Hoodia sp.*

The genus *Hoodia* (Apocynaceae) is a member of the stapeliads, a group of stem succulents widely distributed in South Africa and Namibia. *Hoodia sp.* are used by the San people of South Africa as an appetite suppressant, thirst quencher, a cure for abdominal cramps, hemorrhoids, tuberculosis, indigestion, hypertension, and as anti-diabetes medicine (72). *Hoodia* plants were included in the more than 1000 species screened by the Council for Scientific and Industrial Research (CSIR), South Africa. The finding of several *Hoodia sp.* compounds having anti-obesity activities has resulted in more than 20 patents, including on the active compound responsible for the appetite suppressant activity, 3β-[β-D-thevetopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranosyloxy]-12β-tigloyloxy-14β-hydroxypregn-5-en-20-one (P57 or P57A53), which is a minor compound in *Hoodia* extract (Fig. 2). Appetite suppressant properties were found in *H. gordonii* and *H. pilifera* (73), but P57 was also identified in *H. currorii, H. ruschii,* and *H. parviflora* (74). Intracerebroventricular (i.c.v) injection of P57 in rats resulted in reduced food intake by 50-60%, but no effect was found when P57 was intraperitoneally injected, suggesting that this compound acts on the central nervous system. Despite of the presence of the 4-ring core and 14-OH substitution, no interaction with Na/K-ATPase as the suspected target for cardiac glycosides was found. However, there was an increase of hypothalamic ATP content following P57 treatment. The authors presumed that the P57 mechanism of food intake inhibitor is probably via an intervention of ATP sensitive-nutrient and energy sensing activity in the hypothalamus (75). However, reports regarding the safety of long term administration of *Hoodia* extract are still missing.

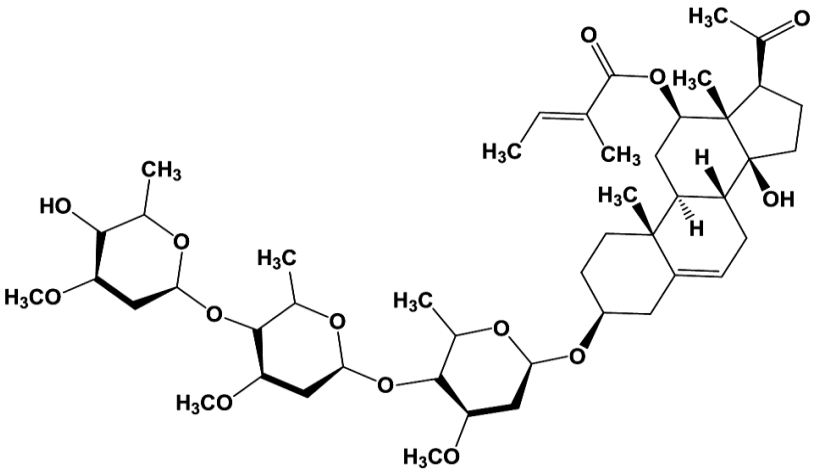


Figure 2. Appetite suppressant from *Hoodia* sp: 3β-[β-D-thevetopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranosyloxy]-12β-tigloyloxy-14β-hydroxypregn-5-en-20-one (P57or P57A53).

2. *Benincasa hispida*

Benincasa hispida (Cucurbitaceae) is widely consumed as vegetable or as ingredient to make fresh drinks and candy in tropical countries, especially in India and Pakistan. Intraperitoneal injection of the fruit methanol extract in male Swiss albino mice caused decrease in food intake but no significant difference in gastric emptying was found between control mice and extract treated mice. At 7th hour after intraperitoneal injection of the extract, food intake was reduced 27%, 38%, and 54% with extract dose of 0.2, 0.6. and 1.0 g/kg body weight respectively (76). The authors suggested that this extract suppresses food intake by targeting central appetite regulation, this is supported by a previous report that *Benincasa hispida* extract showed anti-depressant activity (77) probably by a mechanism similar to a serotonin reuptake inhibitor. However, side effects and body weight loss following administration of this extract were not reported, thus the safety and efficacy of this botanical are questionable.

3. *Mitragyna speciosa*

Mitragyna speciosa (Rubiaceae) is an alkaloid-rich plant fromThailand which leaves have been traditionally used for wound healing, to cure coughing and diarrhea (78). The main alkaloid is mitragynine (Fig. 3). It was predicted that this plant has anti-diabetic activity although no research had been performed to confirm this. The authors presumed an anorectic effect of this plant extract and a secondary effect of reducing the blood glucose level. Total alkaloids extract of young leaves was intraperitoneally injected in male Wistar rats. Acute intraperitoneal administration of 45 and 50 mg extract/kg reduced food intake similar to the positive control (40 mg/kg imipramine). The chronic intraperitoneal administration of the extract at 40 mg/kg dose for 60 days also resulted in lower food intake and smaller weight gain compared to saline-treated rats. For both acute and chronic administration of the extract, water intake was also reduced (78). Although there is no further report to confirm whether mitragynine is the responsible active compound, the author proposed the central targeting mechanism of these activities, supported by previous work on pure mitragynine which showed an interaction with central opioid (79), adrenergic (80), and serotonergic (81) systems in mice. Adverse effects of the long treatment period were not reported in this study. However, this plant might be abused due to its euphoric ('coca like') effect and is illegal in Thailand and Australia. Some symptoms like nausea, vomiting, diarrhea, anorexia, weight loss, hyperpigmentation and psychosis were reported among *Mitragyna* users (82).

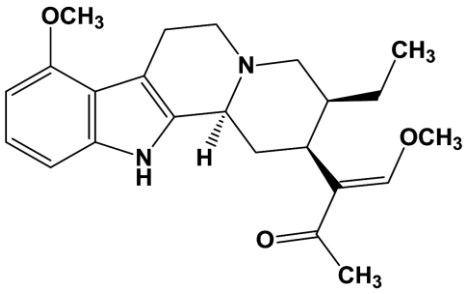


Figure 3. Mitragynine, the main alkaloid in *Mitragyna speciosa* with anorectic effect.

4. *Carraluma fimbriata*

Carraluma fimbriata or *Caralluma ascendens* (Asclepiadaceae), an edible succulent cactus, is indigenously used as a famine food, appetite suppressant and thirst quencher among the tribal population in Western India. It is also consumed as vegetable in the Kolli Hills of South India, or preserved as pickles and chutney in the arid regions of Andhra Pradesh (83). A double blind, placebo controlled, randomized trial with 62 volunteers (age of 25–60 years, body mass index (BMI) greater than 25 kg/m2) was conducted (84). Two capsules, each contains 500 gram powder of 40% alcohol extract, were given daily for 60 days. Maltodextrin capsules were used as placebo. The result showed that the treated group had greater weight loss (2.5%) compared to placebo (1.3%). The other anthropometric parameters such as BMI, waist, and hip circumferences were significantly decreased in the experimental group. The food intake was not directly measured quantitatively in this study, but the appetite behavior was measured by using visual analogues scales (VAS) for 'hunger', 'thoughts of food', 'urge to eat', and 'fullness of stomach'. At day 60, in the treated group, the 'hunger levels' mean value was significantly lower than the placebo group, but there was no significant difference observed in the change of 'thought of food', 'feeling of fullness' and 'urge to eat' between the 2 groups. The experimental group showed a significant reduction in energy and macronutrient intake at the end of the study period. Interestingly, there was a decrease in the intake of cereals, roots and tubers, sugars and sweets, eggs and meat products in the experimental group, while the intake of fruits, vegetables and fish was unchanged. No significant changes in biochemical parameters, such as blood sugar, cholesterol, or triglyceride level, were found. Mild symptoms of the gastrointestinal tract such as abdominal distention, flatulence, constipation and gastritis, were reported in 24% of the experimental group subjects and 20% of the placebo subjects (84). The main phytochemical contents of *Caralluma* are glycosides, saponins, and flavonoids. The responsible compounds for appetite suppressant activity might be ascribed to pregnane glycosides similar to P57, the active compound from *Hoodia* species which is also a pregnane glycoside (84).

Eleven pregnane glycosides from *Carraluma fimbriata* have been isolated (85). More investigations are needed to identify whether one of them is responsible for the activity and to elucidate the mechanism of action. The fact that the experimental group had a lower intake of sugar and sweets for example, indicates that the reward circuit interruption is involved in the activity (79). A standardized extract of *Caralluma fimbriata* is commercially available (Slimaluma^{TM}, Gencor Pacific Group) and has been patented.

5. *Catha edulis*

Catha edulis (Celastraceae) or its local name 'khat', is widely found in East Africa and south-western Arabia where traditionally most of people have a habit to chew the fresh leaves because of its stimulating effect, besides it is also used to cure melancholia, depression, and historically it was used by soldiers and messengers to suppress hunger and fatigue (86). After chewing khat, the sensation of hunger of 6 subjects was decreased while fullness was increased compared to control but no significant changes in ghrelin and PYY level were observed. The alkaloid cathinone (Fig. 4), the main active ingredient with a structure related to amphetamine, was positively correlated with fullness and negatively with hunger (87). It was also reported that chewing the khat leaves for 2 hours significantly delayed the gastric emptying of a radio-labeled semi-solid meal in humans. That cathinone is the active compound is supported by the unpublished *in-vitro* experiment that cathinone causes relaxation of the rat stomach (88). There are no further reports which explain the possible central or peripheral anorexic mechanism of khat. Some papers reported the adverse effect of chewing khat, such as insomnia, hyperthermia, mydriasis and endocrinological disturbances (89). Cathinone may cause cardiovascular complications, increased blood pressure and heart rate via noradrenaline (norepinephrine) release from peripheral neurons similar to the effects of amphetamine (86, 90).

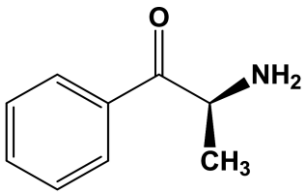


Figure 4. Cathinone, alkaloid from *Catha edulis* with anorectic effect.

6. *Capsicum annuum*

Capsaicin (Fig. 5), the main pungent compound of hot red pepper, is commonly used as food ingredient. A study on its effect on food intake was conducted by including red pepper into a standardized breakfast meal (% energy: protein 18, fat 39, carbohydrate 43) and appetizer (% energy: protein 15, fat 29, carbohydrate 56). Food consumption, desire to eat, hunger, fullness and satiety were measured by using VAS. It was found that red pepper fortification in breakfast meals decreased protein intake, fat intake, and desire to eat in the subsequent meal while satiety and fullness was not significantly changed (91). The authors assumed that this effect correlated with sympathetic nervous system activation in the presence of capsaicin (91). A similar study was performed with 30 subjects who were used to eat spicy foods, to assess whether the decrease on energy intake was due to a sensory or gastrointestinal satiety effect of capsaicin, because in the first study capsaicin was given orally as red pepper. In this study, capsaicin was given in a capsule form which was swallowed with 200 mL tomato juice, or capsaicin was incorporated in 200 mL tomato juice (92). The result showed that the reduction in energy intake was related to a change in food choices since the carbohydrate-rich foods and less-fat rich food consumption were preferred by the subjects, while weight of food intake was unchanged, but the satiety sensation was increased (92).

However, red pepper was not effective to maintain body weight after moderate weight loss. Moderately overweight subjects were treated with a very low energy-diet to aim a body-weight loss of at least 4 kg per 4 weeks. Subjects were divided into 2 groups for the 4 months of weight-maintenance phase; subjects received 135 mg/day capsaicin in capsule and placebo. As a result, there was no significant different in the rate of body-weight regain between 2 groups (93). Capsaicin-containing lunch was also found not to affect satiety, energy expenditure, and plasma PYY concentration, but increased plasma GLP-1 while plasma ghrelin tended to decrease (94).

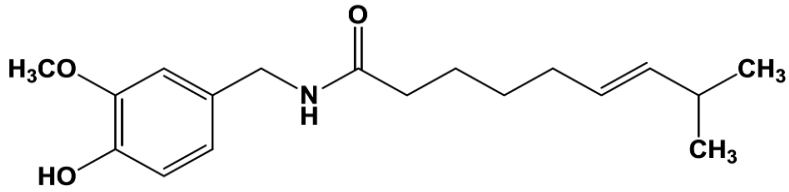


Figure 5. Capsaicin, the main pungent compound from *Capsicum annuum.*

7. *Garcinia cambogia*

The dried fruit rind of *Garcinia cambogia* (Guttiferae) has been used in many Southeast Asian countries as food preservative, flavoring, and carminative. Recently it was introduced to the market worldwide as dietary supplement for weight loss (95). The primary acid principle in the fruit rind is (−)-hydroxycitric acid (HCA), which was found to be up to 30% of fruit rind weight (Fig. 6) (96). Experimental animals fed by HCA-containing *Garcinia cambogia* extract showed suppressed appetite and body fat accumulation. There are several suggestions for the mechanism. Hydroxycitric acid has been reported as a competitive inhibitor of ATP-citrate lyase, the enzyme catalyzing the extra mitochondrial cleavage of citrate to oxaloacetate and acetyl-CoA. Thus, maintaining the acetyl-CoA stock for biosynthesis of fatty acid and cholesterol during the hyperlipogenic nutritional state due to high carbohydrate intake. By inhibiting this enzyme, HCA is suggested to divert carbohydrates and fatty acids into hepatic glycogen, which will be followed by satiety signaling to the brain, resulting in suppression of appetite (96). Several authors found that mice fed by HCA-containing diet had significantly lower insulin and leptin level but the body weight gain, fat pad weight, and serum glucose level were not affected. Besides, serum total cholesterol, triglycerides and non-esterified fatty acid levels of the treated mice were found to be lower than in the control mice. It is suggested that the suppression effect on serum insulin is mediated via a leptin-like activity (97-99). It was also previously reported that HCA increases serotonin release in rat brain cortex *in-vitro* (100). This finding is further supported by the more recent report that HCA inhibited the time dependent uptake of serotonin similar to the well-known serotonin receptor re-uptake inhibitors fluoxetine

and clomipramine. The theory that an increase of serotonin brain level takes part in appetite suppression may provide information on the mechanism of appetite suppression induced by HCA (101).

The same authors reported that no acute oral toxicity, acute dermal toxicity, primary dermal irritation and primary eye irritation were observed in their study, and in their unpublished results it was shown that HCA-SX supplementation over 8 weeks increases serum serotonin levels significantly in human volunteers, showing good bioavailability of HCA. It is not detected in the brain, suggesting that the use of HCA will not give a side effect on the CNS (98). High dose of HCA-containing *Garcinia cambogia* extract (102 mM HCA/kg diet and higher) caused potent testicular atrophy and toxicity (95). Administration of *G. cambogia* extract at recommended dose levels for human use does not show any significant adverse effect on serum testosterone and blood parameters (102). The studied extract dose was 1667.3 mg/day equivalent to 1000 mg HCA/day. Commercial HCA preparations are reported to have in average 25 mg HCA/kg/day or less (103).

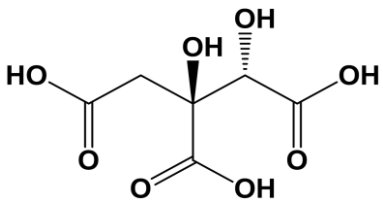


Figure 6. HCA [(−)-hydroxycitric acid], appetite suppressant from *Garcinia cambogia.*

8. *Cyamopsis tetragonolobus*

Guar gum refers to a water soluble galactomannan (Fig. 7), extracted from guar bean (*Cyamopsis tetragonolobus*). Food industry is the major user of this gum, which is applied as thickening or binding agent (E412). Currently, Pakistan and India supply 60% of the world production of guar gum (104).

Several studies demonstrated the ability of guar gum to reduce the appetite in humans (105, 106). It was suggested that the mechanism by delaying the gastric emptying time is most likely, although in another study, the addition of guar gum to a

semisolid meal did not affect gastrointestinal transit time in non-obese human subjects (107). On the contrary, a meta-analysis of randomized trials regarding the efficacy of guar gum to reduce body weight in humans, both published and unpublished, concluded that guar gum is ineffective for reducing body weight and even not recommended as an obesity therapeutic option due to adverse effects (abdominal pain, flatulence, diarrhea, and cramps) (108).

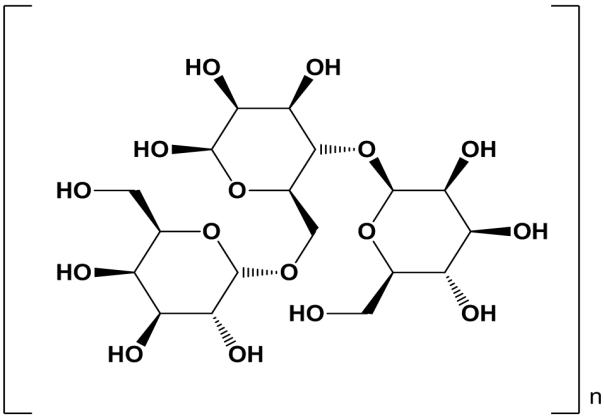


Figure 7. Polysaccharide from *C. tetragonolobus* with galactomannan as a major compound, which may have anti-obesity effect by delaying gastric emptying and delaying abdominal fat absorption.

9. *Amorphophallus konjac*

Similarly to guar bean, *Amorphophallus konjac* (Araceae) root extracts contains glucomannan as a major compound (Fig. 8). This plant is especially found in East Asia and promoted as anti-obesity agent due to its ability to produce satiety sensation and to reduce intestinal fat absorption (109) as cited by Vasques *et al*. (110). However, short administration of *A. konjac* extract to hyperlipidemic type 2 diabetic patients does not result in significant weight loss and food intake (111). A daily administration of *A. konjac* (1.5 g) extract in combination with *G. cambogia* (2.4 g) extract for 12 weeks significantly reduced cholesterol level in obese human subjects but the body weight was not affected (110). Some adverse effects such as flatulence, abdominal pain, esophageal and lower gastrointestinal obstruction were observed.

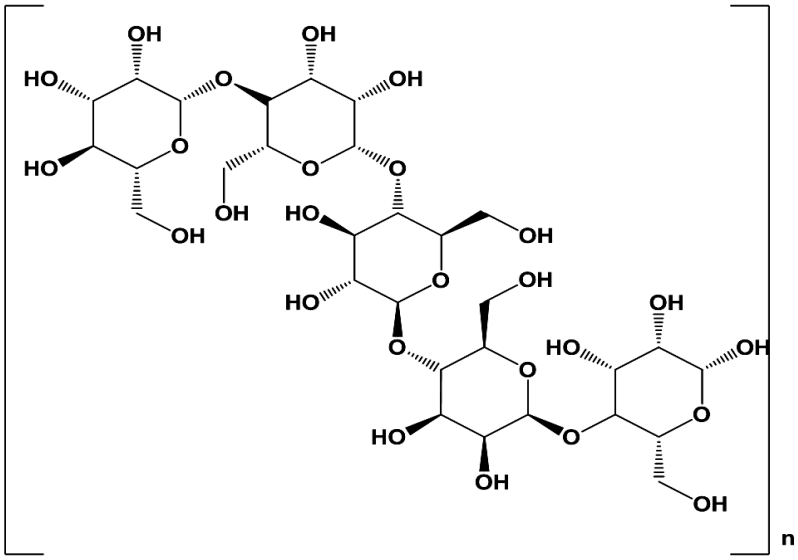


Figure 8. Polysaccharide from *Amorphophallus konjac* with glucomannan as a major compound, which may have anti-obesity effect by delaying gastric emptying and delaying abdominal fat absorption.

10. *Panax ginseng*

Reduction in body weight, food intake, and adiposity was observed after administration of crude saponin extract of red Korean ginseng (Ginseng Radix Rubra) to high-fat diet induced obesity rats and normal rats. This anorexic effect is proposed via the activation of the central appetite regulation pathway, since the reduction in serum leptin level and hypothalamic NPY expression were observed in both groups (112). In a more recent study, protopanaxadiol (Fig. 9A) and protopanaxatriol (Fig. 9B) type saponins from red Korean ginseng were suggested to be the active compounds. More specifically, protopanaxadiol reduced the NPY level of the LHA and PVN, and increased the CCK level of the PVN, compared with the high-fat diet rats, while protopanaxatriol reduced the CCK level of the VMN, suggesting that protopanaxadiol was more effective than protopanaxatriol in reducing appetite. Since the NPY level was only reduced in the LHA and PVN and not in the ARC, it is assumed that protopanaxadiol may not inhibit NPY synthesis in the ARC but inhibit the release of NPY or its transport to the PVN instead (113).

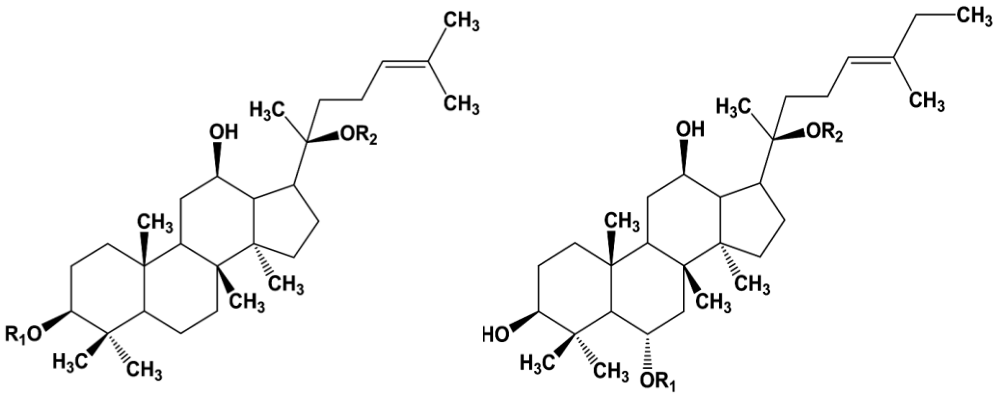


Figure 9. Two types of saponins from red Korean ginseng **A.** Protopanaxadiol type; ginsenoside Rg3 (R1= Glc-(1 – 2)-Glc-, R2=H), ginsenoside Rb1 (R1= Glc-(1 – 2)-Glc-, R2= Glc-(1 – 6)-Glc-), ginsenoside Rd (R1=Glc-(1 – 2)-Glc-, R2= Glc), ginsenoside Rc (R1=Glc-(1 – 2)-Glc-, R2= Ara(*f*)-(1 – 6)-Glc-), ginsenoside Rb2 (R1= Glc-(1 – 2)-Glc-, R2= Ara(*p*)-(1 – 6)-Glc-), ginsenoside Rh2 (R1= Glc, R2= H) **B.** Protopanaxatriol type; ginsenoside Rg1 (R1= Glc, R2= Glc), ginsenoside Re (R1= Rha-(1 – 2)-Glc-, R2= Glc), ginsenoside Rg2 (R1= Rha-(1 – 2)-Glc-, R2= H). Glc = glucose, Ara = arabinose, Rha = rhamnose.

B. Inhibition of nutrient absorption

To reduce energy intake, the digestion and absorption of nutrients should be lowered. Inhibiting fat absorption is the most common target to reduce energy intake since fats contribute more than carbohydrates or proteins to unwanted calories deposition (39). Pancreatic lipase (triacylgycerol acyl hydrolase) is an important lipolytic enzyme synthesized and secreted by the pancreas, which function is to digest dietary triglycerides. The enzyme releases fatty acids from the triglyceride skeleton at the C-1 and C-3 position, and these fatty acids are incorporated into bile acid-phospholipid micelles and further absorbed at the level of the brush border of the small intestine, to finally enter the peripheral circulation as chylomicrons (114). As many as 58 compounds having pancreatic lipase inhibitor activity have been isolated from plant and microbial sources and have been reviewed recently (39). Only a few more recent studies will be mentioned in this review.

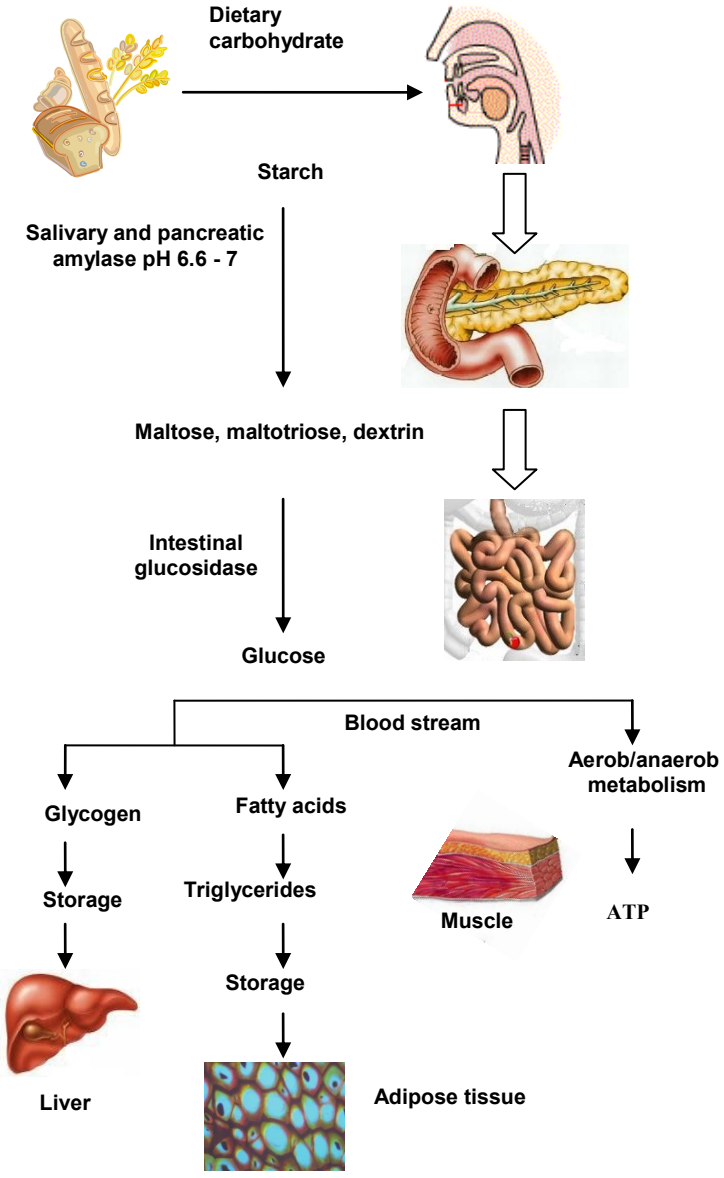


Figure 10. The fate of dietary carbohydrate. Excessive carbohydrate intake leads to an increase of adiposity, therefore the blockage of carbohydrate metabolism by amylase or glucosidase inhibitors might benefit obesity treatment.

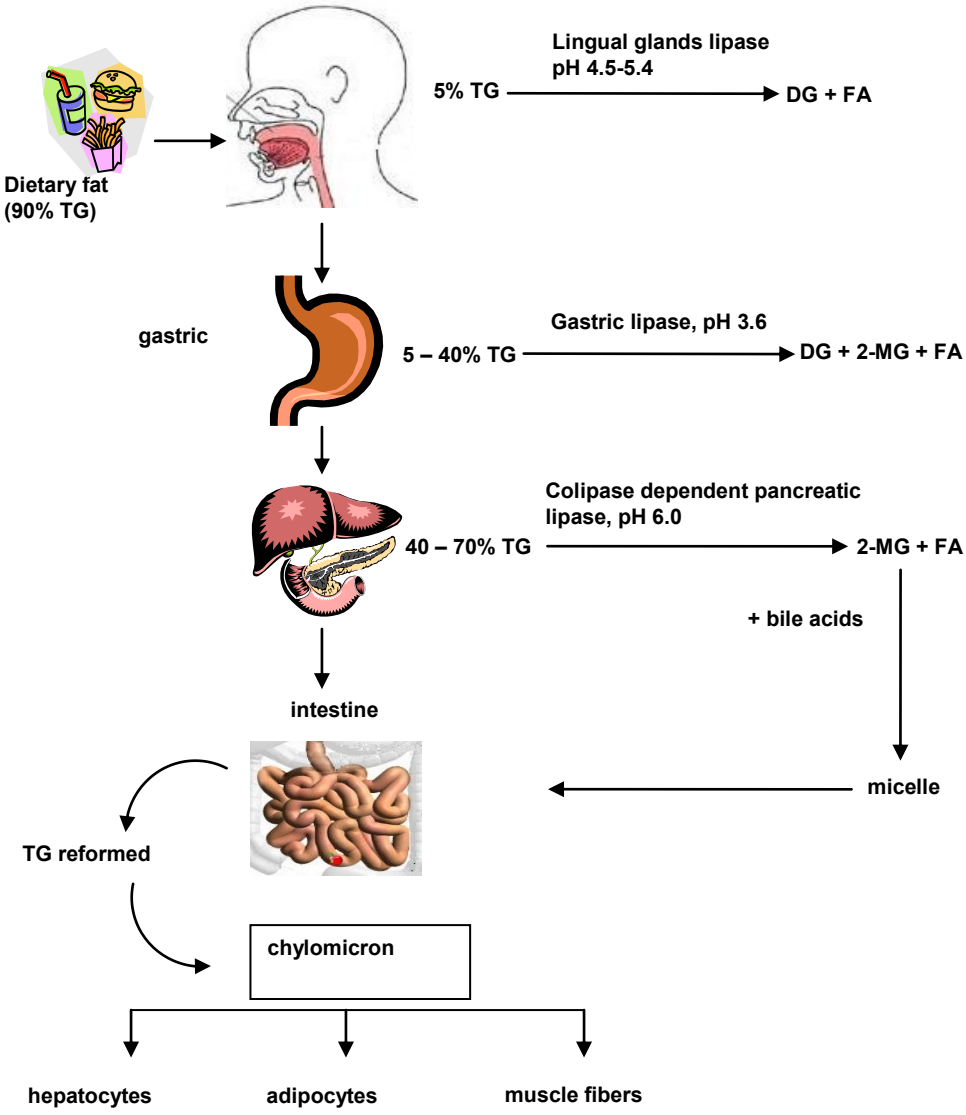


Figure 11. The dietary fat metabolism via multisteps digestion involves different enzymes at different locations. Blockage of one of the pathways may cause decrease of triglycerides reformation leading to reduction in adipocytes differentiation or hepatic triglycerides which might be crucial for obesity treatment. MG monoglycerides, DG diglycerides, TG triglycerides, FA fatty acids.

It is not clear yet if inhibition of protein digestive enzymes is advantageous for obesity treatment since high-protein diets are able to prolong satiety (115). Inhibition of enzymes activity related to carbohydrate metabolism such as α-amylase, maltase, and saccharase, is specifically useful for the treatment of non-insulin-dependent diabetes. But they can be also considered in obesity treatment because usually carbohydrates are the major constituent of human diet. These enzyme inhibitors, which are commercially known as 'starch blockers', delay carbohydrate digestion, reduce postprandial hyperglycemia, therefore reduce the uptake of glucose into adipose tissue and its further conversion into triacylglycerol. The potential of polyphenols from berries as digestive enzymes inhibitors has been recently reviewed (116). Phenolics have a wide spectrum of digestive enzymes inhibition activity especially against α-glucosidases and lipases whereas for proteinases, there is no confirmation whether the activity is only due to non-specific protein binding of tannin-like compounds. Figure 10 and 11 summarize the dietary carbohydrate and fat digestion pathways and where particular enzymes could be blocked by plant derived inhibitors.

Plants which have been reported to have inhibitory activity against human carbohydrase or lipase are the following:

1. *Lagerstroemia speciosa*

The pentacyclic triterpene corosolic acid (Fig. 12), which was isolated from the ethyl acetate extract of *Lagerstroemia* leaves showed uncompetitive α**-**glucosidase inhibitor activity *in-vitro* with IC_{50} 3.53 μg/mL (117). Additionally, alone and in the mixture with *Morus alba* leaves and *Panax ginseng* roots, incorporation of this plant extract into experimental diet at 0.5% of dose induced the expression of rat liver peroxisome proliferator-activated receptor α (*pparα*) mRNA and rat adipose tissue peroxisome proliferator-activated γ (*pparγ*) mRNA (118). Type 2 diabetic patients receiving 32 and 48 mg of *Lagerstroemia* extract (equal to 0.32 and 0.48 mg of corosolic acid) for 2 weeks showed a significant reduction in blood glucose level ($p < 0.001$) as compared to placebo group (119). The change in body weight was not reported in this study.

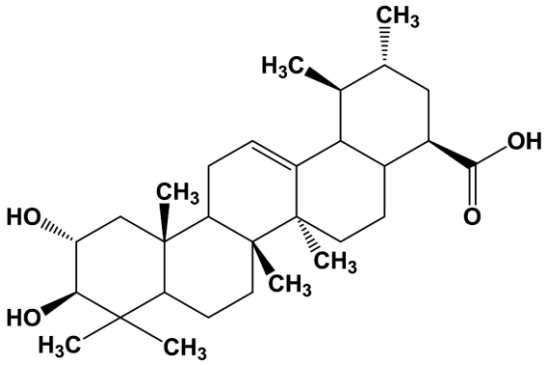


Figure 12. Corosolic acid, a pentacyclic triterpene from *Lagerstroemia speciosa* with α-glucosidase inhibitor activity.

2. *Hibiscus sabdariffa*.

Hibiscus acid and its 6-methyl ester, a lactone form of (+)-allo-hydroxycitric acid (Fig. 13), have been isolated from the methanol extract of commercial *Hibiscus sabdariffa* (roselle) tea which was made from dried flowers. Both major compounds showed a weak inhibitory activity to porcine pancreatic α-amylase *in-vitro* (IC_{50} 3.22 mM and 1.10 M respectively). The activity remained within a 3.5 – 7 pH range (120). There is no further report on the *in-vivo* efficacy of these two compounds.

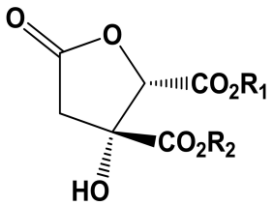


Figure 13. The α-amylase inhibitors from *Hibiscus sabdariffa* : Hibiscus acid (R_1=R_2= H), 6 methyl ester hibiscus acid (R_1= H R_2 = -CH_3).

3. *Nelumbo nucifera*

The anti-obesity effect of ethanol extract of *Nelumbo nucifera* leaves was examined *in-vivo* and *in-vitro*. The extract showed *in-vitro* inhibitory activity on α-amylase and lipase with IC_{50} 0.82 mg/mL and 0.46 mg/mL respectively. Phenolic

compounds were assumed to be the active compounds although there is no further report to support this. Also lipolytic activity in 3T3-L1 adipocytes was observed. *In-vivo*, the inhibitory effect of the extract on the rats pancreatic lipase resulted in a significant decrease of the plasma triacylglycerol level 1 h after oral administration to rats fed with the extract, compared to the untreated controls. The food intake was not affected by the treatment. The body weight, parametrial adipose tissue weight and liver triacylglycerol level were reduced significantly in exercised and extract treated rats, but not in rats treated with exercise only, or in rats treated with extract only. Additionally, also the rats skeletal muscle uncoupling protein 3 (UCP3) was up-regulated only in the combined treatment (121). These results emphasized the importance of drugs therapy in combination with exercise as the more effective obesity treatment.

4. *Phaseolus vulgaris*

Phaseolamin is an α-amylase inhibitor isolated from kidney beans (*Phaseolus vulgaris*). This inhibitor was found to be active only against animal and human amylases (122). It was further discovered that slightly overweight subjects taking tablets with 445 mg (56% w/w) *P. vulgaris* extract before consuming the main carbohydrate-rich-meal for 30 days had significantly greater decrement on body weight, BMI, fat mass, adipose tissue thickness, and waist/hip/thigh circumferences compared to placebo (123). After 9 months of study, the lipoprotein profile of overweight and obese subjects receiving dietary supplement was improved. Low-density lipoprotein and the ratio of low- to high-density lipoprotein decreased and fat excretion in feces increased. Unfortunately, significant levels of antinutritional factors such as lectins and trypsin inhibitors are also present in commercial preparations containing *P. vulgaris* extract (124). The level of antinutritional compounds and the activity of an amylase inhibitor found in several commercial supplements were investigated. It was found that processing the extracts to reduce antinutritional compounds also reduced amylase inhibitor activity to some extent (125). The *in-vivo* efficacy of 3 starch blockers (i.e. *P. vulgaris* extract, *Hibiscus sabdariffa* extract, and L-arabinose extract) on blood glucose level of rats and pigs after starch or sucrose challenge was observed. All were found to have suppressed blood glucose levels after sucrose intervention with L-arabinose as the most effective one, while in case of rice starch, *H. sabdariffa* was the most effective

followed by *P. vulgaris,* whereas L-arabinose had no significant effect. When given in combination, the level of circulating glucose was suppressed both after sucrose or rice starch intervention (126).

The toxicity of Blockal, a commercial starch blocker containing a standardized *P. vulgaris* extract was tested. No toxicity symptoms were found after oral administration of 2500 mg/kg body weight of the extract to rats (127). On the other hand, some studies failed to show the effectiveness of starch blockers in delaying glucose or insulin response to a meal in humans. No marked differences were observed in blood insulin and glucose level between the test meal containing commercial starch blockers and placebo. A breath hydrogen test was used to measure undigested dietary carbohydrates since this method is sensitive to small amount of carbohydrates (128). There was no significant different of breath hydrogen level between the two groups. This implicates that all carbohydrates consumed with the test meal were completely digested. Furthermore, *in-vitro* experiments showed that maltase and glucoamylase were capable to hydrolyze starch in the presence and absence of these starch blockers (129).

5. *Triticum aestivum*

The obese women consumed weight reduction regiment (1000 kcal/day) which contain an expanded-whole wheat protein product for 12 weeks had a significant greater weight loss (5.5 kg) than the isocaloric standard low-calorie diet control group (2.8 kg, p=0.05) (130). The suggested mechanism is via α-amylase inhibition, which was confirmed by the more recent report where α-amylase inhibitor preparation isolated from wheat protein was infused into human duodenum. The concentration needed to inhibit 90% of amylase activity *in-vivo* was 4.5 mg/mL extract, while *in-vitro* 4 mg/mL was needed to inhibit 75% of amylase activity. In spite of the decrease in amylase activity, the level of plasma glucose and several hormones (e.g. insulin, c-peptide, glucagon, gastric inhibitory polypeptide, neurotensin, peptide YY) concentrations were not affected. This was due to the fact that the amylase inhibitor from wheat and white bean only affect postprandial glucose and insulin, while in this study no carbohydrates were infused to the intestine before intervention with inhibitors. Pancreas secretion of lipase, trypsin, chymotrypsin, and bile acids was also unaffected. This is important since

the amylase inhibitors of wheat have homology with the trypsin inhibitor, and prolonged pancreatic protease inhibition might stimulate pancreas carcinogenesis. The K_i value of the inhibitor of wheat was 57.3 nM, while the T_{50} (temperature giving 50% inactivation after 30 minutes incubation) was 88.1^{o}C (131, 132). Because of this high activity and thermal stability, this purified wheat inhibitor has potential for obesity treatment.

6. *Morus alba*

In several countries such as India, Pakistan, and Thailand, *M. alba* leaves has been traditionally used to cure diabetes. Glucosidases inhibitor activity of the leaf extract has been reported by several authors. *In-vitro* disaccharidase inhibitor activity of ethanol extract of *M. alba* leaves in human and rat intestine was examined (133). The extract, which contained 0.24% 1-deoxynojirimycin (Fig. 14) showed similar strong inhibition of sucrase, maltase, and isomaltase, in both human and rat small intestine. Only for rat small intestine K_i values were mentioned: 21, 25, and 45 μM for sucrase, maltase and isomaltase, respectively. *In-vivo*, when administered together with sucrose, the extract suppressed the rat blood glucose level but the suppression level depends on the ratio of extract to sucrose.

Some reports support the wider use of *M. alba* as a potent source of anti-obesity drugs (134-136). Type 2 diabetic patients taking *M. alba* leaves powder packed in a capsule cause a more pronounced decrease in serum cholesterol, triglycerides, free fatty acids, LDL- and VLDL-cholesterols, lipid peroxides, erythrocyte membrane lipids and membrane lipid peroxidation than the positive control glibenclamide (134). The potential of *M. alba* hot water extract to be consumed as an anti-diabetic herbal tea was reported. The brewing time of 3 – 5 minutes for tea preparation was found to be the most optimum compared to the longer ones (7, 10, and 30 minutes) since the maltase and sucrase inhibitor activity of *M. alba* leaves tea, *in-vitro*, was the highest (137). A strong correlation between the level of 1-deoxynojirimycin content and α-glucosidase inhibitory activity of *M. alba* leaves was found (135). Apart from previously described glucosidases inhibitor activity of *M. alba*, rats fed with high-fat diet together with the mixture of *M. alba* leaves*, Melissa officinalis* (Linn.) leaves, and *Artemisia capillaries* (Thunb.) var. arbuscula Miquel leaves aqueous extract were reported to have lower

levels of serum triglycerides, total cholesterol, adipose tissue mass, and body weight gain than mice fed with high-fat diet alone. *Morus alba* extract in this mixture was standardized according to 1-deoxynojirimycin content. There was no difference of food intake in both groups. The authors proposed that the upregulation of expression of mRNA encoding PPAR-α hepatic target enzymes was responsible for those results (136). This is in accordance with the above mentioned study (118).

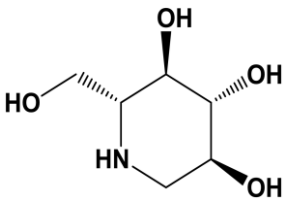


Figure 14. 1-deoxynojirimycin, glycosidase inhibitor from *Morus alba*.

7. *Panax ginseng* and *Panax quinquefolius*

Besides *Panax ginseng* roots, apparently ginseng berries are considered to be at least equivalently potent. A crude ginseng berry extract induced mice body weight reduction but not the root when injected intraperitoneally (138). Saponin extract from both root and berry were found to suppress mice body weight gain and plasma triacylglycerol level when orally administered. The proposed mechanism is via inhibition of pancreatic lipase leading to inhibition of intestinal dietary fat absorption (139). The efficacy of this botanical was tested in C57BL/KsJ db/db mice, which has obese and diabetic phenotypes because of disruption of the leptin receptor, and their lean littermates. Intraperitoneal injection of *P. ginseng* berry extract (150 mg/kg body weight) reduced fasting blood glucose levels significantly in *db/db* mice but not in lean mice. The body weight of both groups was significantly decreased (140). Furthermore, it was found that ginsenoside Re (Fig. 9B), the major steroidal saponin in ginseng, is correlated with the ginseng hypoglycemic effect but not correlated with body weight, food intake, and energy expenditure. Daily administration of 6 g ginseng extract improved plasma glucose and insulin profiles in humans but the body weight was not affected (141). The other possible responsible compounds for the last activities were not

identified. The gastrointestinal tract is considered as the action site of the ginseng postprandial hypoglycemic effect (142). Apart from inhibition of carbohydrates digestive enzymes, there is another mechanism described for hypoglycemic activity of American ginseng roots (*Panax quinquefolius*) namely by improving beta cell insulin production and protecting these cells from apotopsis (143).

In another study, crude saponin extract from stem and leaves of *P. quinquefolius*, containing 9 major ginsenosides (Rg1, Re, Rg2, Rb1, Rc,Rb2, Rb3 and Rd, Fig. 9A-9B), was tested *in-vitro* for pancreatic lipase inhibition. At 0.5 mg/mL dose, the ginsenosides Rb1, Rb2, Rc, and Rd showed strong inhibition almost similar to orlistat at a dose of 0.008 mg/mL, with Rc being the most active. Oral administration of crude saponin in a lipid emulsion (1 g/kg body weight) inhibited the increase of rat plasma triacylglycerol level compared to lipid emulsion only. When incorporated into rat high-fat diet at 1% and 3% dose, crude saponins suppressed parametrial adipose tissue weight compared to high-fat diet control but body weight and food intake were not different (144). American ginseng was found to be effective to improve blood glucose level in normal and type-2 diabetic patients but effect on body weight was not reported (145, 146).

8. *Aframomum meleguetta* and *Spilanthes acmella*

Ethanol extracts of two native African plants, *Aframomum meleguetta* and *Spilanthes acmella*, were both tested for inhibitory activity against human pancreatic lipase *in-vitro* in 0.75 – 2.0 mg/mL concentration range. *Aframomum meleguetta* seed extract (90% at 2.0 mg/mL) showed higher inhibition than *S. acmella* flower bud extract (40% at 2.0 mg/mL) (147). However, no further work on the identification of the responsible compounds and *in-vivo* experiment has been reported.

9. *Salix matsudana*

The polyphenol fraction of *Salix matsudana* leaves was tested for lipase and α-amylase inhibitory activity. *In-vivo*, after oral administration of the extract, there was a significant decrease in rat plasma triacylglycerol (lipid emulsion + 570 mg/kg body weight extract dose), parametrial adipose tissue and body weight, hepatic total cholesterol content, and diameter of adipose tissue (high fat diet + 5% extract dose)

when compared to control (high-fat diet or lipid emulsion only). Feces fat content also increased while food intake was unaffected. *In-vitro*, the polyphenolic extract acted synergistically with norepinephrine to induce lipolysis at a concentration of 1 mg/mL. The extract was also found to inhibit α-amylase activity at a concentration of 250 – 2500 µg/mL and the incorporation of palmitic acid into brush border membrane vesicles at concentrations of 500 and 1000 µg/mL. The responsible compounds were elucidated as apigenin-7-*O*-D-glucoside which inhibits α-amylase, luteolin-7-*O*-D-glucoside and chrysoeriol-7-*O*-D-glucoside (Fig. 15) which inhibit palmitic acid incorporation into small intestine brush border membrane vesicles. All compounds induced lipolysis synergistically with norepinephrine like the crude extract (148, 149).

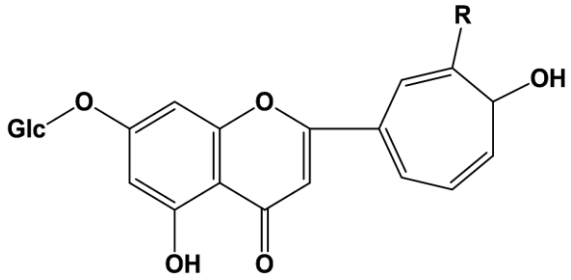


Figure 15. Lipase and α-amylase inhibitors from *Salix matsudana*: Apigenin-7-*O*-D-glucoside (R=H), Luteolin-7-*O*-D-glucoside (R=OH), Chrysoeriol-7-*O*-D-glucoside ($R=OCH_3$).

10. *Glycyrrhiza uralensis*

Licochalcone A isolated from *Glycyrrhiza uralensis* roots showed weak non-competitive lipase inhibitory activity *in-vitro* with *K*i value 32.8 µM (Fig. 16). Although weaker than orlistat, the inhibitory activity of this compound is reversible (150).

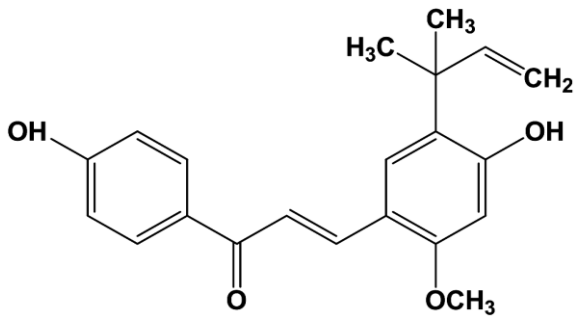
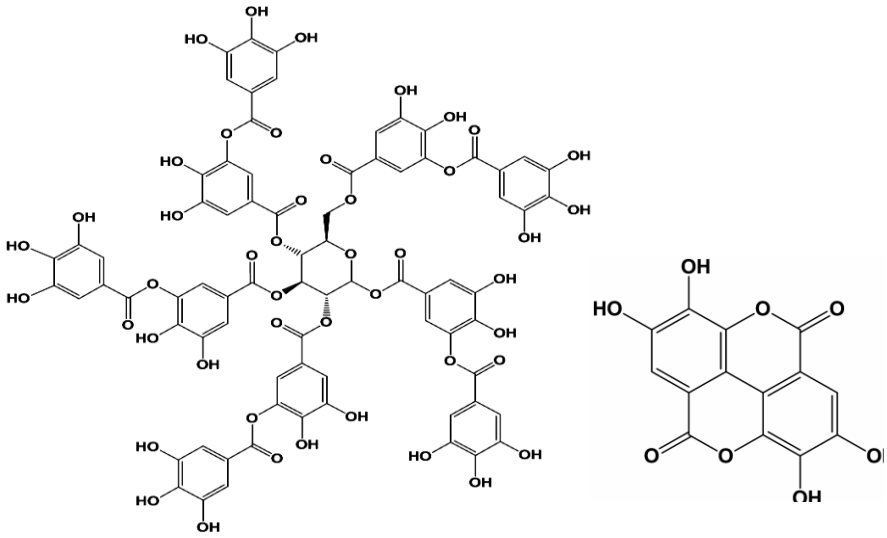


Figure 16. Licochalcone A, a weak non-competitive lipase inhibitor from *G. uralensis.*

11. *Punica granatum*

The body weight gain of high-fat diet induced obese mice given the *Punica granatum* leaves extract by gavage at 800 mg/kg dose for 5 weeks was suppressed compared to obese control mice, also final adipose pad weight, serum glucose, triglyceride, total cholesterol, and high-density lipoprotein cholesterol were reduced. Food intake was lower in extract treated obese mice, similar to Sibutramine treated obese mice, but not in treated normal mice. Furthermore, after oral administration of lipid emulsion, extract treated obese and normal mice had a lower level of serum triglycerides and total cumulative triglycerides absorption but the normal mice had lower triglycerides absorption. *In-vitro*, the extract showed inhibition of pancreatic lipase activity almost to 100% inhibition at 0.1 mg/mL concentration, this was confirmed *in-vivo* by an increase of fecal fat secretion. Tannic acid (Fig. 17A) and ellagic acid (Fig. 17B) were thought to be responsible for the activity (151).



A. **B.**

Figure 17. Lipase inhibitors from *Punica granatum*: **A.** Tannic acid **B.** Ellagic acid.

2. Blocking adipogenesis

Recently the adipocyte cell has been positioned not only as a passive centre of energy storage (in triglycerides form) during excessive nutrient availability, but also as active centre of energy mobilization when required. Various bioactive molecules are secreted from adiposity tissue as mediators of unrelated biological processes (152). Some of them are strongly related to obesity, such as satiety, energy homeostasis, blood pressure, hepatic and peripheral glucose homeostasis (152). Therefore, adiposity mass and size are included as important markers of obesity, both are driven by two individual processes: differentiation, i.e. the formation of new adipocyte cells from precursor cells, and an increase of adipocyte size (hypertrophy) as a result of a positive energy balance (153). Many anti-obesity plant screenings are targeting on these observations, particularly adipocyte differentiation. Exploration of adipocyte differentiation should at least include lipogenic capacity and size of cytoplasmic lipid droplets, insulin sensitivity and glucose uptake, and secretion of various biomarkers. Adipocyte differentiation is a highly regulated complex process where the changes in expression level of approximately 300 proteins are involved, most of these changes could also be observed at transcription factors level (152, 154). The best studied are peroxisome proliferator-activated receptors (PPARs) and CCAAT-enhancer-binding proteins (C/EBPs) (152). The PPARs are members of the nuclear receptor family and thought to be important mediators for lipid metabolism and glucose homeostasis (155). The γ subtype of PPAR, or PPARγ, is highly expressed in adipose tissue. It was found that during conversion of preadipocytes into mature adipocytes, *pparγ* mRNA expression was induced earlier as well as at higher level than other PPARs, and together with other transcription factors such as C/EBPa and C/EBP homologous protein 10 (CHOP-10), suggesting that PPARγ activation is crucial for adipocytes development and function (156). Recent studies indicate that many other transcriptional factors are involved in adipocyte differentiation as has been reviewed by several authors (152, 154, 157). Figure 18 summarizes the adipogenesis network involving several recently discovered transcriptional factors.

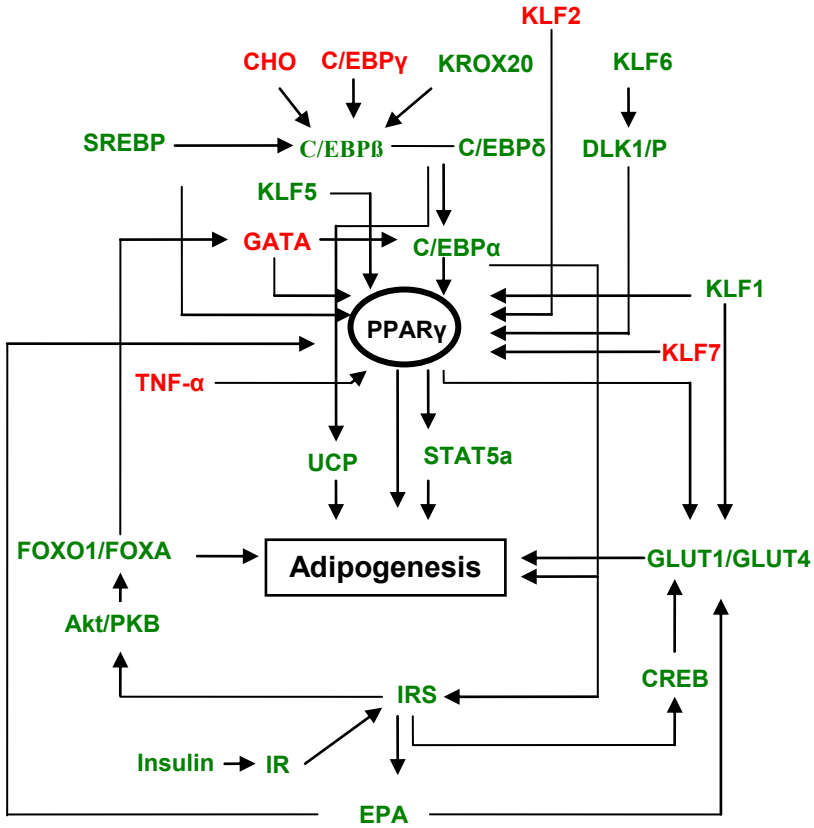


Figure 18. Some important transcriptional factors involved in adipogenesis regulation network. Green = activation, red = suppression. PPARγ acts as a master regulatory factor directly or indirectly driven by hundreds of transcriptional factors. EPAS1 (Endothelial Per-ARNT-Sim 1), IR(Insulin Receptor), IRS (Insulin Receptor Subunit), CREB (cAMP Response Element Binding), Akt/PKB (AKT/Protein Kinase B), FOXO1 (Forkhead box 1), FOXA (Forkhead A), GLUT (Glucose Transporter), UCP (uncoupling protein), STAT (Signal Transducers and Activators of Transcription), TNF- α (Tumor Necrosis Factor), KLF (Krüppel Like Factor), PPARγ (peroxisome proliferator-activated receptor γ),C/EBP (CCAAT-enhancer-bindingproteins),GATA 2/3 (Globin Transcription Factor 2/3),DLK1/PREF1 (Delta Like 1/Pre-Adipocyte Factor1), CHOP (C/EBP homologous protein), KROX (Zinc finger protein), SREBP 1c (sterol regulatory element binding protein-1c).

Several plants have been reported to be able to block the adipogenesis process via suppression or activation of transcription factors.

1. *Hibiscus sabdariffa*

In-vitro anti-adipogenic activity of aquaeous *Hibiscus sabdariffa* flower extract has been studied. Adipogenic differentiation is initiated with the conformational

changes of fibroblast-like preadipocyte to a round shape. The extract added at the onset of differentiation and 4 days after induction of differentiation was found to block accumulation of the lipid droplets in insulin and dexamethasone induced adipocyte differentiation of 3T3-L1 preadipocytes. From Western Blot analysis, the authors concluded that this activity was correlated with the suppression of (C/EBP)α and PPARγ expression at protein level (158). Oral administration of 120.00 mg/kg/day of standardized *H. sabdariffa* calyces aqueous extract (containing 33.64 mg of total anthocyanins) significantly reduced body weight gain in MSG-induced-obese mice but not the food intake. However, since the alanine aminotransferase (ALT, one of the markers of tissue damage) level was significantly increased, further study on the toxicity is required (159).

2. *Panax ginseng*

Wild ginseng roots (*Panax ginseng*) extract, orally administered to leptin deficient mice at 100 and 200 mg/kg body weight, dose-dependently decreased the body weight and blood glucose level compared to the control (160). The proposed mechanism was via activation of PPARγ and lipoprotein lipase in adipose tissue since the expression of related mRNA was found to be increased. Similarly, the expression of the glucose transporter 4 (GLUT4) and insulin receptor (IR) targeted mRNA in the skeletal muscle and liver were increased, thus the mechanism of the observed hypoglycemic effect is probably by improving insulin resistance and glucose utilization. Additionally, the adipose droplet size was found smaller in treated rats (160). The dose of 500 mg/kg body weight wild ginseng ethanol extract administered together with high fat-diet significantly inhibited mice body weight gain, reduced diameters of white adipose tissue (WAT) and brown adipose tissue (BAT), fasting blood glucose, triglyceride, and free fatty acid levels, and *glut4* mRNA expression in a dose dependent manner. Interestingly, food intake was reportedly increased and physical activity decreased in treated mice (161). A typical ginseng saponin glycoside, ginsenoside Rh2 (Fig. 9A), was found to effectively inhibit adipocyte differentiation via PPARγ inhibition. The PPARγ expression was significantly blocked by ginsenoside Rh2 treatment. Rosiglitazone-induced PPARγ transcriptional expression was significantly downregulated, representing an antagonistic activity of ginsenoside Rh2 to PPARγ.

Ginsenoside Rh2 also inhibited 3T3-L1 adipocytes differentiation via the activation of AMPK, which was further confirmed by induction of 2 molecular markers associated with AMPK signaling pathway, carnitine palmitoyltransferase (CPT)-1 and uncoupling protein (UCP)-2. A similar pathway was also found to be activated by ginsenoside Rg3 (Fig. 9A) (162), while a cAMP dependent pathway might be involved in the lipogenesis blocking activity of Rb1 (Fig. 9A) and Rg1 (Fig. 9B) (163).

3. *Rosa canina*

Aqueous acetone extracts from the fruit (50 mg/kg/d) and seeds (12.5 and 25 mg/kg/d) of *Rosa canina* (rose hip) administered to mice suppressed the gain of body weight and visceral fat weight. Plasma triglyceride and free fatty acid levels were significantly reduced on the 14th day while food intake was unchanged. Plasma glucose level increase after glucose loading was also averted.

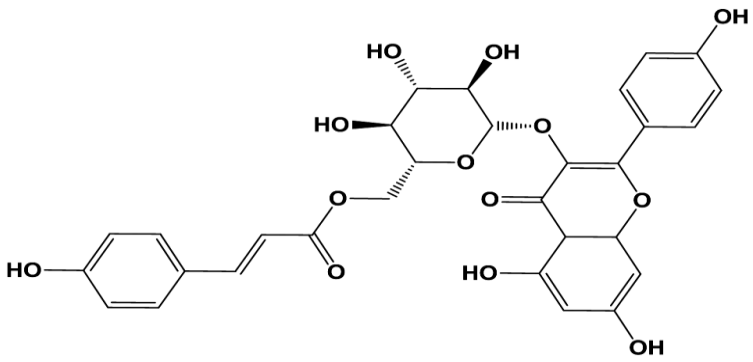


Figure 19. *Trans*-tiliroside, compound from *Rosa canina* targeting lipid metabolism acceleration and glucose homeostasis improvement.

The responsible active compound was isolated and identified as *trans*-tiliroside (Fig. 19) which showed stronger effect than orlistat. Oral administration of *trans*-tiliroside reduces the expression of *pparα* mRNA levels in liver tissue suggesting lipid metabolism acceleration and glucose homeostasis improvement as targets of this compound. No toxic effect was reported (164), however, there is no further report on the anti-obesity activity of this plant in humans.

4. *Pinus densiflora*

Water extract of *Pinus densiflora* (pine needle) was incorporated in the normal mice diet and high-fat diet at 1% dose. As a result, the body weight gain and visceral fat mass of both treated groups was significantly lower than control. The food intake was not significantly different. Plasma triglyceride and cholesterol levels were also reduced in treated groups. Plasma leptin level which was increased in high-fat diet control group was returned to normal level in treated group, but the glucose level was unchanged in all groups. Supplementation of the extract at 25 μg/mL, 100 μg/mL, and 500 μg/mL into a 3T3-L1 adipocytes, significantly suppressed adipocyte differentiation, reduced glycerol-3-phosphate dehydrogenase (GPDH) activity, and expression of *pparγ* mRNA but did not affect triglyceride level (165).

5. *Camellia sinensis*

Healthy subjects receiving a green tea extract containing 50 mg caffeine and 90 mg epigallocatechin gallate (EGCG, Fig. 20), had significant higher energy expenditure (EE), and lower respiratory quotient (RQ) than subjects receiving placebo or 50 mg caffeine. There was no difference in protein oxidation rates (shown from insignificant urinary nitrogen excretion), but urinary norepinephrine excretion was higher in the green extract subjects. Fat and carbohydrate oxidation contribution to 24-h EE was higher in the green tea group than in placebo group. This effect could not be attributed to the green tea caffeine content since the given dose was lower than the minimum threshold for thermogenesis stimulation (166). Exolise® (Arkopharma Laboratories, Carros, France) is a capsul containing AR25® (Frutarom Switzerland Ltd., Wädenswil, Switzerland and Burgundy Botanical Extracts, Reyssouze, France), an 80% ethanol dry green tea extract standardized at 25% catechins expressed as EGCG. *In-vitro*, AR25® noticeably reduced gastric lipase and to a lesser extent, pancreatic lipase activity, and lipids emulsification. The effective dose was 60 mg AR25/g triolein, which corresponds to a reasonable daily intake (1,500 mg) and dietary fat consumption of food restricted subjects (30–50 g/day) (167). *In-vivo*, 70 subjects receiving AR25 capsules equivalent to 270 mg EGCG per day showed a significant decrease of body weight and waist circumference after 3 months of treatment, but changes in plasma cholesterol levels were insignificant. No significant adverse effects were observed such as an increase in

heart rate, a common limitation of sympathomimetic drugs used as thermogenic agents (168). The effect of EGCG and other related catechins from green tea on body weight loss, food intake and endocrine system was studied (169). Only EGCG showed a marked effect on food intake. Rats treated with 80 mg EGCG/kg body weight showed a 50% decrease in food intake after 2 days of treatment. The mechanism was thought to be independent from the change in leptin level since the leptin receptor defective obese Zucker rats also responded to EGCG. Further observations to elucidate the anorexic effect were performed by measuring several related plasma peptides level, such as adrenocorticotropic hormone (ACTH), NPY, corticotropin-releasing factor (CRF), urocortin, and galanin, in male Sprague Dawley rats after they were treated with 83 mg EGCG/kg body weight for 2 days, but no obvious changes were observed. An insignificant increase of serum aspartate aminotransferase and γ-glutamyltranspeptidase activity in treated rats might be related to the anorexic effect and needs further confirmation. The EGCG effect on body weight loss, hormone level changes, and food intake was more effective when administered by intraperitoneal injection, suggesting that EGCG is not absorbed efficiently into the body when given orally. Additionally, EGCG induced some changes in endocrine systems, which might relate to inhibitory properties of EGCG on human prostate and breast tumor growth.

In another study, green tea was given to rats instead of drinking water together with commercial laboratory chow (170). The amount of green tea was similar to that of human consumption. Green tea treatment did not affect the body weight, food and water intake, but the treated rats showed significant loss of adipose tissue and lipid metabolism improvement. A marked decrease in the uptake and translocation of GLUT4 in adipose tissue were thought to be involved in the loss of adipose tissue weight, since it reduces the conversion of the incorporated glucose into fatty acids and triglyceride. Another possible mechanism is by suppressing the expression of PPARγ and its downstream molecule, SREBP1, thus suppressing adipocyte differentiation. The activity of hormone-sensitive lipase (HSL) in adipose tissue was also increased 1.3-fold. Green tea also significantly decreased total-, free-, HDL- and LDL-cholesterol and FFA in the plasma. A similar mechanism was mentioned in a different study (171). Epigallocatechin gallate in purified form, Teavigo™ from DSM Nutritional Products (94% purity), 0.5% and 1% (w/w) incorporated into the high fat chow was found to

reduce the rats body weight and body fat gain while food intake was not altered. Feces energy content was slightly increased, suggesting lower intestinal fat absorption. Furthermore, the expression of UCP-2 and some enzymes related to glucose catabolism and lipogenesis such as glucokinase, stearoyl-CoA desaturase-1 (SCD1), and malic enzyme in different tissue were observed. Malic enzyme activity in chicks was found to decrease as a response to high-fat-diet treatment (172). There was no change in BAT UCP2 and SCD1 expression. Marked alteration on related enzymes was found in liver including an increase on UCP2 expression and decrease on expression of glucokinase, SCD1 and malic enzyme. The UCP 2 expression was reduced in skeletal muscle. Leptin and SCD1 were reduced in WAT. Acute extract administration resulted in decrease of respiratory quotient (RQ) mainly during feeding phase, while energy expenditure and body temperature were unchanged.

After a four week very low energy diet intervention which resulted in 5 – 10% body weight loss in overweight and moderately obese subjects, consumption of green tea containing caffeine (104 mg/d) and catechins (573 mg/d, of which 323 mg was EGCG) was not effective for body weight maintenance (173). A comprehensive review on green tea anti-obesity activity has been published (174).

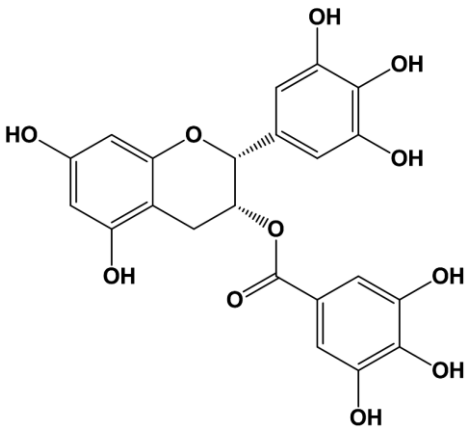


Figure 20. Epigallocatechin gallate (EGCG), the main catechin in green tea which shows anti-obesity activity probably by increasing energy expenditure and suppressing adipocytes differentiation.

7. *Zingiber mioga*

Mioga (*Zingiber mioga*) is a Japanese traditional edible spice. The phosphate-buffered saline soluble part of mioga ethanol extract, exerted *in-vitro* anti-differentiation activity in 3T3-L1 adipocytes at 500 µg/mL dose. This was marked by a decrease of glycerol-3-phosphate dehydrogenase activity and triglyceride accumulation in a culture. *In-vivo*, mioga extract orally administered to mice at 50 mg/mouse/d reduced the body weight gain compared to control and 10 mg treated mice but the food intake was not significantly different in all groups. The epididymal fat weight was lower in mice with treatments compared to control. Epididymal fat is commonly used to study the effect of different hormones (such as leptin and insulin) and dietary macronutrients (fat and carbohydrate) on specific enzymes activities, such as lipoprotein lipase, an enzyme responsible for the breakdown of circulating triglycerides in chylomicra and very low-density lipoproteins (175). There are no further reports regarding identification of active compounds, though the authors presumed that the compounds are hydrophylic and not phenolics such as anthocyanins (176). The immature flower buds or young shoot of this plant are commonly consumed in Japan as fresh condiment or blanched vegetable (177), but in this study it was not mentioned which part of the plant was used.

8. *Zingiber officinale*

Oral administration of fresh *Zingiber officinale* (ginger) rhizome ethanol extract (200 mg/kg in 2% gum acacia) to cholesterol-fed induced hypercholesterolemia rabbits significantly reduced the levels of serum and tissue cholesterol, serum triglycerides, LDL- and VLDL-cholesterol, serum phospholipids and increased HDL-cholesterol levels when compared with the group only receiving acacia gum (178). The extract was also found to exhibit a significant antihyperglycaemic and lipid lowering activity when orally administered to streptozotocin-induced diabetic rats. The level of serum total cholesterol and triglycerides was decreased while the level of HDL-cholesterol was increased as compared to untreated diabetic rats (179). Similar results were obtained when the water extract was administered intraperitoneally (180). *In-vivo*, administration of 0.25 g and 1 g/kg body weight extract after oral administration of lipid emulsion reduced the rise of mice plasma triacylglycerol level. Incorporation of extract to experimental diet at 1% and 3% dose reduced body weight gain in high-fat treated

mice compared to control, as well as parametrial adipose tissue weight, but energy intake was not affected. Although excretion of triacylglycerol was not significant among high-fat diet treated mice, the mechanism was thought to be inhibition of intestinal dietary fat absorption which was confirmed by *in-vitro* experiments. The extract inhibited hydrolysis of triolein emulsified with phosphatidylcholine by pancreatic lipase inhibitor (181).

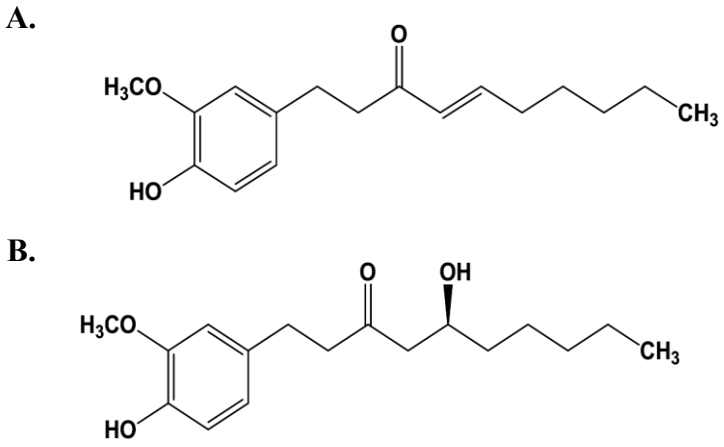


Figure 21. Adipogenesis blockers from *Zingiber officinale* : **A.** 6-shogaol **B.** 6-gingerol.

Oral administration of ethanolic ginger extract exerted hypoglycaemic action in streptozotocin-induced diabetic rats (200 mg/kg – 800 mg/kg dose). At 800 mg/kg dose, the extract was as potent as positive control chlorpropamide at 250 mg/kg dose. The proposed mechanism is similar to chlorpropamide, i.e. by enhancing the release of endogenous insulin from pancreatic ß-cells and facilitating peripheral tissue uptake and utilization of glucose (182). In *in-vitro* experiments, the ginger-derived components 6-shogaol (Fig. 21A) and 6-gingerol (Fig. 21B) showed significant inhibition of downregulation of adiponectin expression mediated by TNF-α in 3T3-L1 adipocytes at 25 μM and 50 μM concentration. To explain the mechanism, it was shown that 6-shogaol, but not 6-gingerol, is a PPARγ agonist which increased the expression of the adiponectin and aP2 expression. In case of 6-gingerol, the pathway was elucidated via activation of c-Jun N-terminal kinase (JNK) as it inhibits the phosphorylation of JNK

and SAPK/ERK-MAP kinase kinase (SEK1/MKK4), which is one of the upstream kinases of JNK (183).

9. *Coix lachrymal-jobi*

The water extract of *Coix lachrymal-jobi* (adlay) seeds was orally injected to the high fat diet rats at the dose of 500 mg/kg body weight for 4 weeks. As result, the body weight and food intake of the treated group were significantly lower than the control group. The sizes and the wet weight of WAT, as well as the wet weights of the, epididymal and peritoneal fat in treated group were significantly lower than the control group. The weight of BAT of treated group tended to be higher than the control group although not significant. The serum lipids profile in the treated group was improved, while the leptin level was decreased. The expression of *tnf-α* and *leptin* mRNA in WAT of treated group were found to be significantly lower than the control group (184). There is no further report on efficacy of this plant in humans.

10. *Lagerstroemia speciosa*

The extract of *Lagerstroemia speciosa* (Lythraceae) leaves, or banaba, inhibited the differentiation of 3T3-L1 adipocytes and stimulated glucose uptake in 3T3-L1 adipocytes via PPARγ and GLUT4 pathways (185). In another study, by bioassay guided fractionation, three active elligatannins; lagerstroemin and flosin B (Fig. 22A), and reginin A (Fig. 22B), were found to be active in increasing glucose uptake level in rat adipocytes (186).

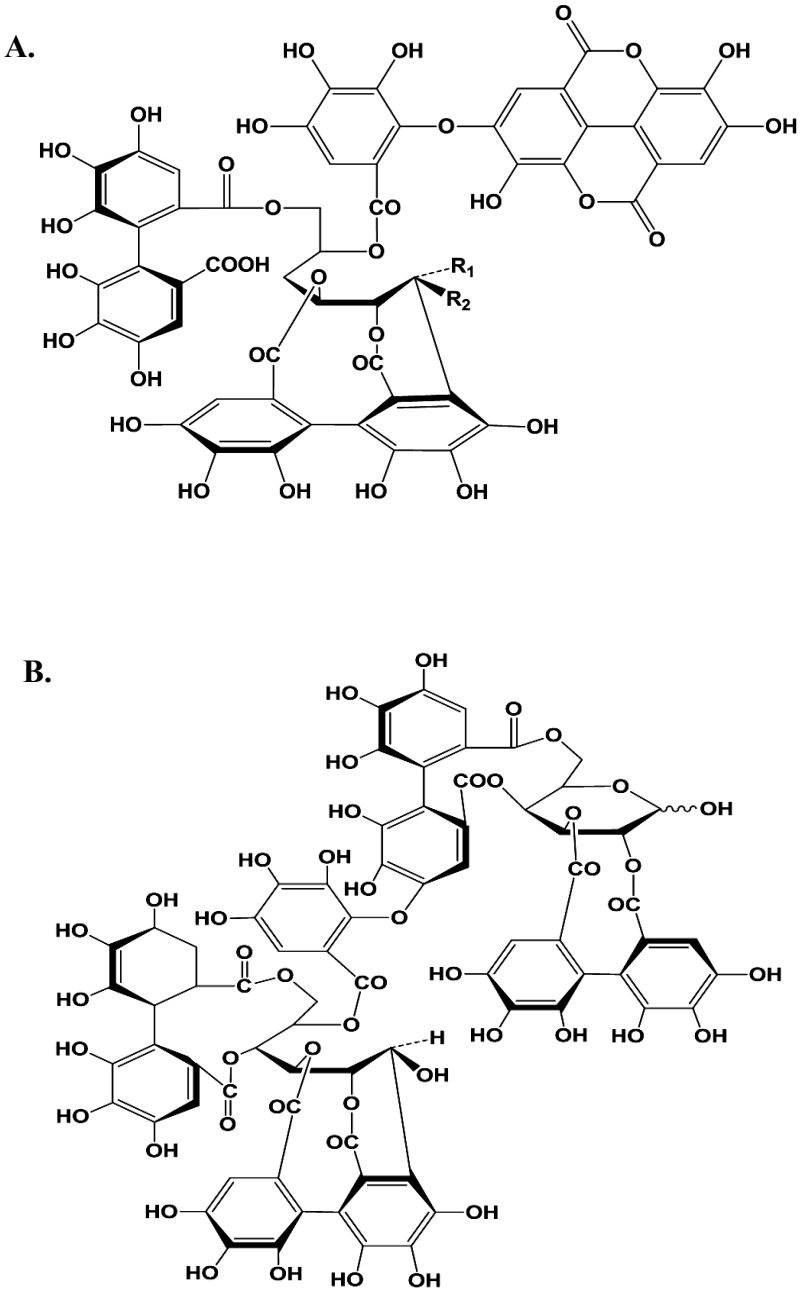


Figure 22. Elligatannin isolated from *Lagerstroemia speciosa* inhibits adipocyte differentiation probably by increasing glucose uptake level in adipocytes: **A.** Lagerstroemin (R1= OH, R2= H), Flosin B (R1= H, R2= OH) **B.** Reginin A.

3. Energy expenditure stimulation

Energy expenditure is centrally and pheripherally regulated; neural pathways which activate thermogenesis and pheripheral pathways in which energy is produced by oxidation of stored fats (187). In neural pathways, some markers for thermogenesis are closely interrelated to food intake regulation such as melanocortin receptor (MCR), melanine-concentrating hormone (188), and leptin (187). Pheripherally, when energy intake is greater than energy expenditure, most of the excess calories are stored as triglycerides (TG) in white adipose tissue (WAT) (189).

Lipolysis takes place as a response to energy demand where TG in WAT is hydrolyzed into free fatty acids and glycerol. Stimulation of TG hydrolysis leading to mobilization of stored fat can be a choice to combat obesity but this step should be followed by oxidation of the newly released fatty acids (190). Triglycerides mobilization is a highly regulated process involving several facilitators at molecular level, which is summarized in Figure 23. Catecholamines, insulin, and natriuretic peptide, are the three main mediators in the human lipolytic pathway (189). Catecholamines (adrenaline, noradrenaline) mediates human WAT lipolysis via lipolytic ß-adrenoceptors and antilipolytic α2-adrenoceptors (190). Insulin is lipolytic inhibitor acting via insulin receptors. Natriuretic peptide, a lipolytic agent specific for primate fat cells, activates guanylate cyclase via non-GPCR dependent pathways (191). To a lesser extent, other possible pathways involved are TNF-α induced lipolysis which is important at basal level, as well as signaling pathways of nicotinic acid-, prostaglandin-, and adenosine receptors (189).

The subsequent downstream lipolytic pathways are regulated by two important enzymes; hormone sensitive lipase (HSL) which is predominant under stimulated conditions (192) and adipose triglyceride lipase (ATGL) active in basal lipolysis (193). Other WAT enzymes such as triacylglycerol hydrolase are also found to play a role (194). Hormone sensitive lipase activity is regulated via phosphorylation and translocation mechanisms (195), while ATGL expression is upregulated by fasting and glucocorticoids (193).

Several non enzyme proteins are indirectly involved in lipolysis regulation by interacting with HSL, e.g. lipotransin which is able to fit the enzyme's active site to the

lipid droplet surface, thus improving the activity (196), and perilipin that blocks the enzyme access by covering the adiposity lipid droplets (197). Subsequent to lipolysis, free fatty acids and glycerol are transferred from adipocytes into the blood stream and distributed into energy demanding body tissues, a process which is mediated by a passive diffusion mechanism and several facilitating proteins (190).

Adenosine 5'- monophosphate-activated protein kinase (AMPK) is a phosphorylating enzyme important for fatty acid and glucose metabolism. The activation of AMPK leads to the stimulation of hepatic fatty acid oxidation and ketogenesis, inhibition of cholesterol synthesis, lipogenesis, and triglyceride synthesis, inhibition of adipocyte lipolysis and lipogenesis, stimulation of skeletal muscle fatty acid oxidation and muscle glucose uptake, and modulation of insulin secretion by pancreatic ß-cells. In the liver, AMPK activation causes a decrease in fatty acid, triglyceride, and sterol synthesis but an increase in fatty acid oxidation and ketogenesis by phosphorylation of acetyl-CoA carboxylase and and 3-hydroxy-3-methylglutaryl-CoA reductase. The same effects are also obtained when the ratio of insulin-to-glucagon decreases but whether it is an AMPK independent pathway is unclear yet (198). In adipose tissue, AMPK activation targets HSL which leads to inhibition of lipogenesis by phosphorylation of acetyl-CoA carboxylase (199). The physiological function of AMPK depends on the level of activation. Antilipolytic action is generated when AMPK is activated doubly, while apoptosis is observed at higher activation magnitude.

The use of β3-adrenoceptor agonists to accelerate thermogenesis was also proposed (200). The selective β3-adrenoceptor agonists stimulate lipolysis in brown adipose tissue (BAT), leading to an increase of thermogenesis. The potential of these agents as anti-obesity compounds has been reviewed (201, 202). Treatment with CL 316,2439, a β3-adrenoceptor agonist, delayed high fat diet induced obesity in rats. Metabolic rate and mitochondrial uncoupling protein (UCP) both in BAT and WAT were found to be increased while food intake was unaffected (203).

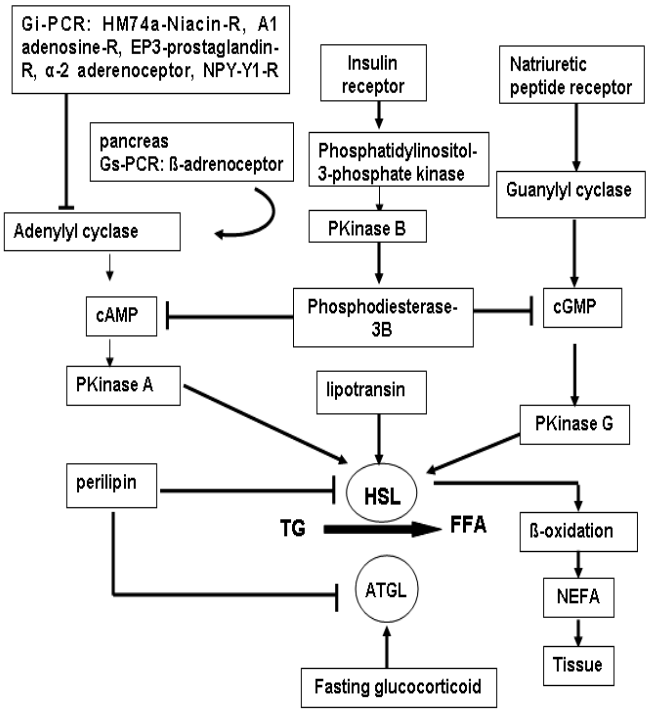


Figure 23. Diagrams of receptors and enzymes/co-enzymes network in lipolysis leading to thermogenesis regulation. Hormone sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) are key regulators for triglyceride hydrolysis into free fatty acids which will further undergo ß-oxidation, resulting in non-esterified fatty acids (NEFA) as a source of energy for requiring tissue. ⊣ = inhibit.

In rodents, β3-adrenoceptors are found to be highly expressed in WAT and BAT, while in humans only in BAT (204). In the past, it was well accepted that BAT is prominent in rodents and infant humans but is rapidly lost during postnatal development (205). Therefore the interest in the development of anti-obesity drugs targeting on BAT stimulation has dropped. However, recent findings unexpectedly revealed the presence of this tissue in adult humans as recently reviewed (206). Recent studies reported that the adult humans BAT is metabolically active (207, 208), suggesting a possible role of

BAT in the regulation of thermogenesis and body fat content. Based on the observation of 2-[^{18}F]fluoro-2-deoxyglucose (FDG) uptake into BAT, it was found that the amount of BAT is higher in the same subjects at winter as compared to summer, and when the same subjects were exposed to cold condition as compared to the warm condition. BAT was also found to inversely correlate with BMI, total and visceral fat of the subjects.

Despite whether BAT is a feasible target for anti-obesity in humans needs further investigation, several studies on plant extracts having thermogenesis stimulator activity via this pathway have in fact been published.

1. *Vitis vinifera*

The health promoting properties of *Vitis vinifera* (grape) is believed to be due to its phenolics content. Resveratrol, a phytoalexin found in red wine and grapes (Fig. 24A), was found to increase basal energy expenditure and adaptive thermogenesis in high fat-fed mice treated with resveratrol at the daily dose of 2 or 4 g/kg diet. An increase in mitochodria size and activity was observed in the treated mice The body weight gain and fat content of the treated group was also lower then the control group while the food intake was unchanged. There were no changes in hepatic toxicity parameters and water intake, indicating that resveratrol treatment was well tolerated by the mice at the given dose (209).

Resveratrol down-regulates the expression of *pparγ*, *c/ebpα*, *srebp1*, *fas*, *lpl*, and *hsl* mRNA in 3T3-L1 adipocytes. While *sirt3*, *ucp1*, and *mfn2* mRNA expression was up-regulated (210). In another study, C57BL/6NIA mice were fed with high calorie diet supplemented with resveratrol. Resveratrol markedly altered 144 out of 153 pathways in high fat diet mice towards those of standard diet. Some of them are important markers to prolong lifespan when downregulated or upregulated, such as upregulated insulin sensitivity, downregulated insulin signaling, downregulated IGF-1 and mTOR signaling, downregulated glycolysis, upregulated AMPK and PPARγ coactivator 1α(PGC-1α) activity, upregulated Stat3 signaling, and upregulated mitochondrial number (211). The resveratrol doses used in this study were 5.2 and 22.4 mg/kgdiet/day which are a reasonable amount for daily human consumption. Resveratrol content of Italian red wine and grape juice ranged between 2 – 6 μg/L and

0.2 – 0.3 µg/L, respectively (212), and the recommended safe dose for humans is 5 – 10 mg/d (213).

There are some reports on anti-obesity related activities of other grape and grape seeds derived compounds, such as procyanidin and vitisin (Fig. 24B). The reported activities concern the inhibition of adipogenesis by *in-vitro* methods (214-217).

Only few studies reported the relevance and potential of grape seeds or its related compounds for obesity treatment in humans. In a randomized, placebo-controlled, double-blind, cross-over study, subjects who received 300 mg grape-seeds extract supplement (containing > 90% procyanidines) showed no difference in 24 h energy intake with the placebo in the total study population. Only subjects whose energy requirement was lower than the median of 7.5 MJ/day have 4% less 24 hour energy intake compared to placebo (218).

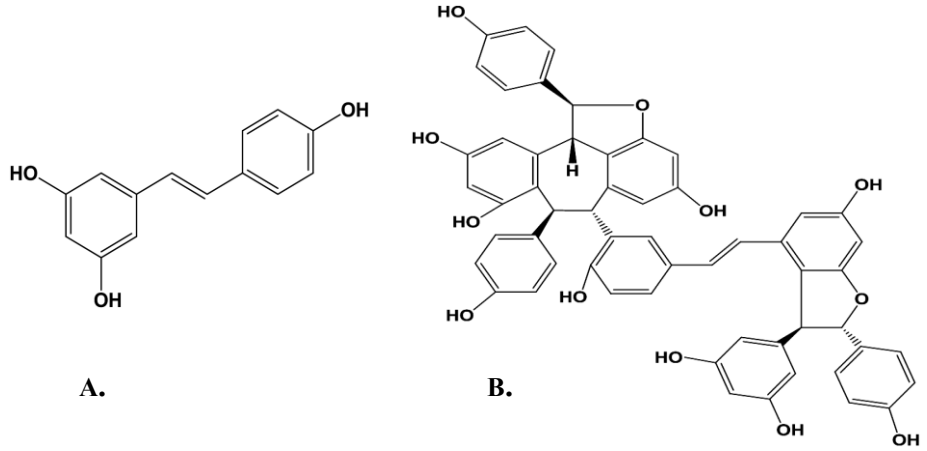


Figure 24. Adipogenesis blocker from *Vitis vinifera*: **A.** Resveratrol **B.** Vitisin A.

It was reported that daily consumption of 480 mL of Concord grape juice for 12 weeks did not lead to significant weight gain in overweight subjects, but consumption of polyphenol-free grape-flavored drink did (219). In another study which involved overweight and obese subjects who regularly consumed 20–30 g alcohol/day, consumption of an iso-caloric diet (1500 kcal/day) with 10% of energy either from white wine or grape juice showed a significant body weight reduction. There was no placebo group in this study (220).

From the available data, it is too early to recommend grape seeds extract or compounds derived from it as a new therapy to reverse obesity in humans. The animal studies may reveal an activity on SIRT1, the well-known mammalian sirtuin, or other human energy expenditure related pathways, but whether a reasonable dose allows the absorption of an effective level of the active compounds needs to be investigated further.

2. *Citrus sp.*

Obese subjects consuming *Citrus paradise* (grapefruit) juice or grapefruit capsules or half of fresh grapefruit before each meal three times a day lost body weight more than placebo (221). The mechanism is not known yet. Sinetrol is a citrus-based polyphenolic dietary supplement. It is a mixture of *Citrus sinensis* (sweet orange), *Citrus aurantium* (bitter orange, Seville orange), *Citrus paradise*, and *Paullinia cupanna* (guarana). Overweight and obese subjects consuming 350 mg of Sinetrol extract daily for 12 weeks had more pronounced body weight and body fat loss than placebo. The mechanism was proposed to be through induction of lipolysis since the extract exhibited strong phosphodiesterase inhibition (97%), stronger than caffeine (56%), at 0.01% concentration. The activity was assumed to be due to the synergistic effect between polyphenolics present in the extract such as cyanidin, naringin, naringenin, narirutin, and hesperidin (present at 5–10% in Sinetrol, Fig. 25A – 25C) (222).

Herbal preparations containing *Citrus aurantium* fruit/rind extract are commercially available as weight loss promoting agents. These preparations are usually sold as “ephedra free” preparations. This is as a response to the FDA ban of *Ephedra* containing products in 2004 because of the side effects. The active principle, the sympathomimetic amines synephrine (phenylephrine) and octopamine are structurally similar to epinephrine and norepinephrine (Fig. 25D – 25E) (223). Synephrine and octopamine are selective ß-3 adrenoreceptor agonists which stimulate cAMP production (224, 225). Dried fruits, extracts, and commercial *C. aurantium* products contain significant amounts of synephrine, but octopamine content might be too low to consider being involved in activity (226). Subjects consuming capsules containing 26 mg of synephrine increased energy expenditure by 29%. No effect on pulse rate and blood

pressure was observed (227). Several reports point to possible toxicity problems. Oral administration of 2.5-20 mg/kg *Citrus aurantium* alcoholic extracts, which contain 4% and 6% active principal synephrine, significantly reduced food intake and body weight gain in rats. However, 10% - 50% mortality and myocardium toxicity was observed in the experiments. This finding is a serious contra-indication for the use of bitter orange, and specifically synephrine as a potent substitute of *Ephedra* containing products (228). Tachycardia symptoms were reported on woman treated with 50 μg thyroxine daily for hypothyroidy, after taking *Citrus aurantium* at the daily dose of 500 mg of extract (equal to 30 mg synephrine daily) (229). A systematic review revealed 7 randomized controlled clinical trials of products containing *Citrus aurantium* articles in the period of 1966 – 2004 (of which only one satisfied the authors' criteria) found no evidence that herbs containing *Citrus aurantium* are effective for weight loss (230). Similarly, another review mentioned that the available information is insufficient to support the efficacy or safety claims of *Citrus aurantium* (226).

By using HPLC method, it was found that the content of synephrine is 0.10–0.35% in the dried fruit, 3.00–3.08% in dried extract, and 0.25–0.99% in commercial slimming preparation (231). The synephrine recommended effective safe dose found in previous reports has been reviewed, which varies between 100 – 300 mg/day, which more or less equal to 34 – 100 g of dried fruit/day (232). This should be taken into consideration although the culinary use of *C. aurantium* is not so common since the fruit is too sour to be consumed as a table fruit, but the fresh or dried ripe fruit is eaten in Iran and Mexico while immature fruits are sometimes pickled and used as a condiment (226). The peel of *C. aurantium* is often used as ingredients in marmalade and beer, and the flower is used in some teas (233).

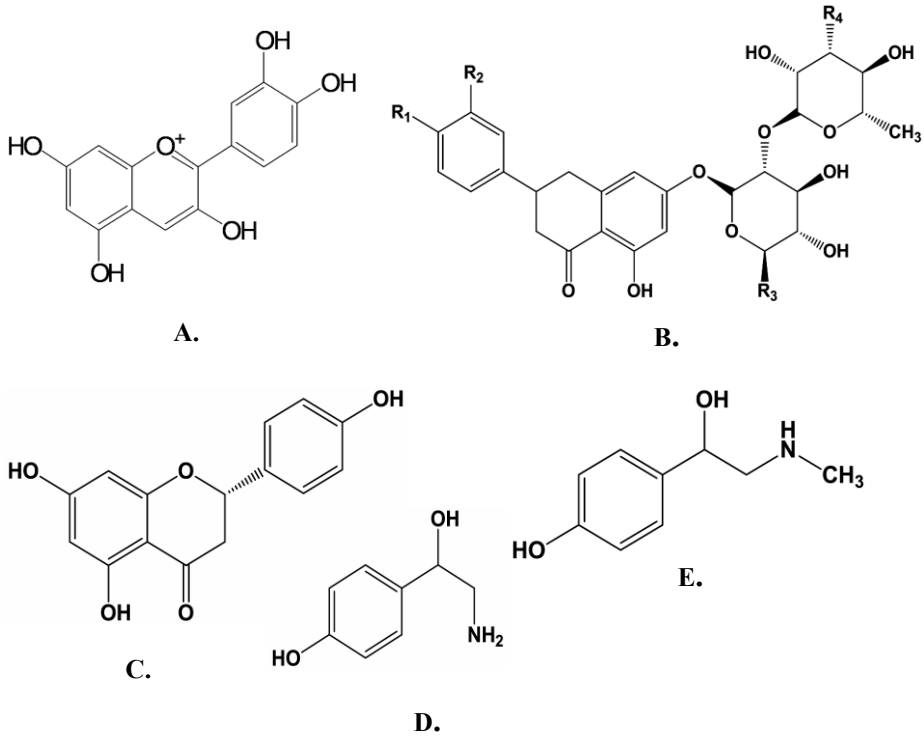


Figure 25. Polyphenolics which induce lipolysis (A – C) and sympathomimetic amines which act as ß-3 adrenoreceptor agonist ligands (D – E) from *Citrus sp.* **A.** Cyanidin, **B.** Naringin (R1= OH, R2= H, R3= -OCH_3, R4= OH), Narirutin (R1= OH, R2= H, R3=OH, R4= OH), Hesperidin (R1= OCH_3, R2= OH, R3= OH, R4= CH_3, **C.** Naringenin, **D.** Synephrine, **E.** Octopamine.

3. *Salvia miltiorrhiza*

Salvia miltiorrhiza (red Sage) is a famous Traditional Chinese Medicine, especially for treatment of coronary heart diseases. The leaves are commonly used as a meat seasoning while its medicinal benefit is attributed most to the root. Cryptotanshinone (Fig. 26A), a diterpene quinone derivative, has been isolated from the dried roots of *S. miltiorrhiza* (234). This compound was found to be a potent AMPK activator in the presence of AMP by *in-vitro* and *in-vivo* experiments. From *in-vitro* experiments using mouse C2C12 skeletal myoblasts cell lines, cryptotanshinone was found to indirectly activate AMPK by reducing intracellular ATP. This AMPK activation effect was also associated with the phosphorylation of AMPKα Thr172, an important intermediate for AMPK activation, and the phosphorylation of ACC Ser^{79}, the

intracellular substrate for AMPK. At 20 μM concentration, cryptotanshinone was more potent than positive controls 5-amino-imidazole-4-carboxamide riboside AICAR (500 μM) and Metformin (2 mM).

In parallel with AMPK activation, cryptotanshinone was also found to be as potent as insulin to facilitate glucose uptake by stimulating translocation of *glut4* to the plasma membrane and *glut1* mRNA expression. Cryptotanshinone exerted intense suppression of *acc1* and *acc2* mRNA expression and a simultant increase in the *cpt-1* mRNA expression, as well as *pgc-1α* and *ucp2* mRNA expression. The authors also conducted an *in-vivo* experiment by using *ob/ob* (C57BL/6J-Lep^{ob}) mice. Oral administration of cryptotanshinone for a month resulted in significant decrease of the body weight at 200, 400, and 600 mg/kg/day dosage and less fat in adipose tissue. At the maximal dosage (600 mg/kg/day), food intake was also reduced. Serum triglycerides and cholesterol levels were reduced, while AMPK activity of the skeletal muscles was higher in the treated group, whereas AMPKα level was only slightly increased. Reduction in blood sugar level was also observed in treated *ob/ob*, *db/db*, and Zucker Diabetic Fatty (ZDF) mice (235).

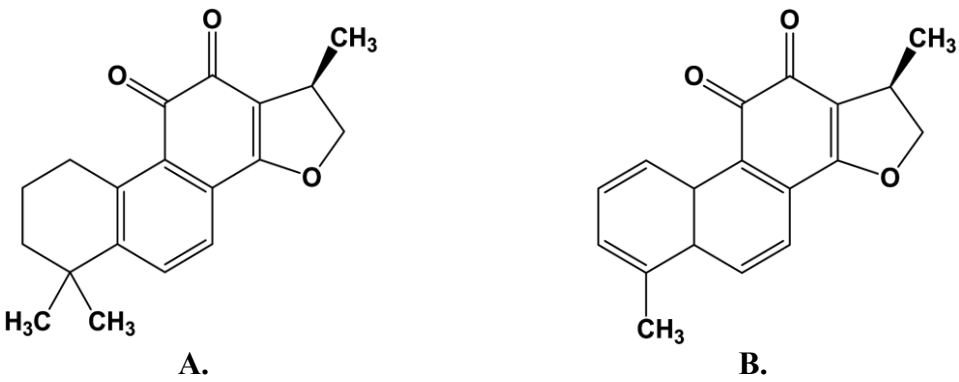


Figure 26. Anti obesity compounds from *Salvia miltiorrhiza*: **A.** Cryptotanshinone, AMPK activator **B.** 15,16-dihydrotanshinone, DGAT inhibitor.

Diacylglycerol acyltransferase (DGAT) is a microsomal enzyme important in the metabolism of glycerolipids. The inhibition of this enzyme was reported to associate with an increase of energy expenditure in mice (236). *In-vitro*, cryptotanshinone and

15,16-dihydrotanshinone (Fig. 26B) isolated from S. *miltiorrhiza* were reported as a weak DGAT inhibitor with the IC50 values of 10.5 µg/mL and 11.1 µg/mL (237). Apart from these *in-vitro* and animal data, there are no reports on the thermogenic efficacy of this herbal in humans.

4. *Camellia sinensis*

Theaflavins (Fig. 27A – 27C) are considered as one of the major active phenolic principal in tea (*Camellia sinensis*). A mixture of the major theaflavins in black tea i.e. theaflavin, theaflavin-3-gallate, and theaflavin-3,3-digallate suppressed intracellular lipid accumulation in HepG2 cells *in-vitro* at 50 µM dose. It increased the phosphorylation level of AMPK Thr^{172} and ACC serine79, and as a result, hepatic fatty acids decreased. Treatment with theaflavins also induced fat oxidation. It was concluded that AMPK activation was involved in the activity of theaflavins (238). Incorporation of oolong tea powder into high fat diet at the dose of 5% has reduced body weight and final parametrial adipose tissue, and the accumulation of liver triglyceride as compared to the high-fat diet-fed rats. From the *in-vitro* data, the authors concluded that this is due to pancreatic lipase inhibitory activity of several compounds present in the tea extract, besides the enhancement of noradrenaline-induced lipolysis in adipose tissue by the caffeine present in the tea extract (239). In another study, 20 g/kg diet of green tea extract added into a high-fat diet did not affect the body weight gain and food intake of experimental rats but prevented the increase in body fat gain induced by high-fat diet which was correlated with the restoration of energy expenditure to a similar level as in the control group. The administration of the β-adrenoceptor antagonist propanolol and the green tea extract into high-fat diet rats inhibited the changes in body weight gain, as well as energy expenditure although not significant (240).

The role of tea catechins as compounds that significantly contribute to anti-obesity activity of tea has been reviewed (241). Epigallocatechin-3-gallate is the most abundant catechin in green tea extract (242). Several studies reported the role of EGCG (Fig. 20) and caffeine (Fig. 27D) present in tea in increasing energy expenditure in humans. Normal subjects that consumed capsulated green tea extract, which equals to 50 mg caffeine and 90 mg EGCG/day, showed significantly higher 24 hour energy expenditure but lower 24 hour respiratory quotient (RQ) than placebo or 50 mg caffeine

group (166). Interestingly, carbohydrate oxidation was significantly lower but fat oxidation was significantly higher in the green tea extract group than the caffeine or placebo group. A similar study was conducted where an oolong tea beverage which was brewed according to normal daily consumption was used. Healthy subjects receiving a full-strength tea (equal to total consumption of caffeine and EGCG 244 mg and 270 mg, respectively) showed siginificantly higher 24 h energy expenditure and fat oxidation than the placebo treated group (only water), but not different with the caffeinated water treated (135 mg caffeine/day) group (243). From the results of these studies it is not clear whether EGCG alone has an activity. In a more recent report describing a randomized double blind, placebo-controlled, cross-over pilot study, the 24 h energy expenditure of overweight/obese subjects receiving 300 mg EGCG was not significantly different with the placebo although fat oxidation was increased (244). A synergy between caffeine and EGCG was previously proposed (245).

The effect of habitual caffeine intake to the effect of green tea – caffeine mixture on overweight/obese subjects was studied (246). It was found that after receiving a green tea-caffeine mixture (270 mg EGCG, 150 mg caffeine) per day, subjects that normally consume relatively much caffeine (> 300 caffeine mg/day) had a greater weight loss, thermogenesis and fat oxidation level than the low caffeine consumer subjects (< 300 caffeine mg/day).

The studies of green tea anti-obesity effect in humans have been reviewed (174, 241). Most of them showed significant decrease in body weight and body fat as compared to baseline or placebo and the underlying mechanism of these effects is on thermogenesis and fat oxidation. However, the authors suggested additional studies with a fixed nutrient, energy intake, and physical activity to confirm these results.

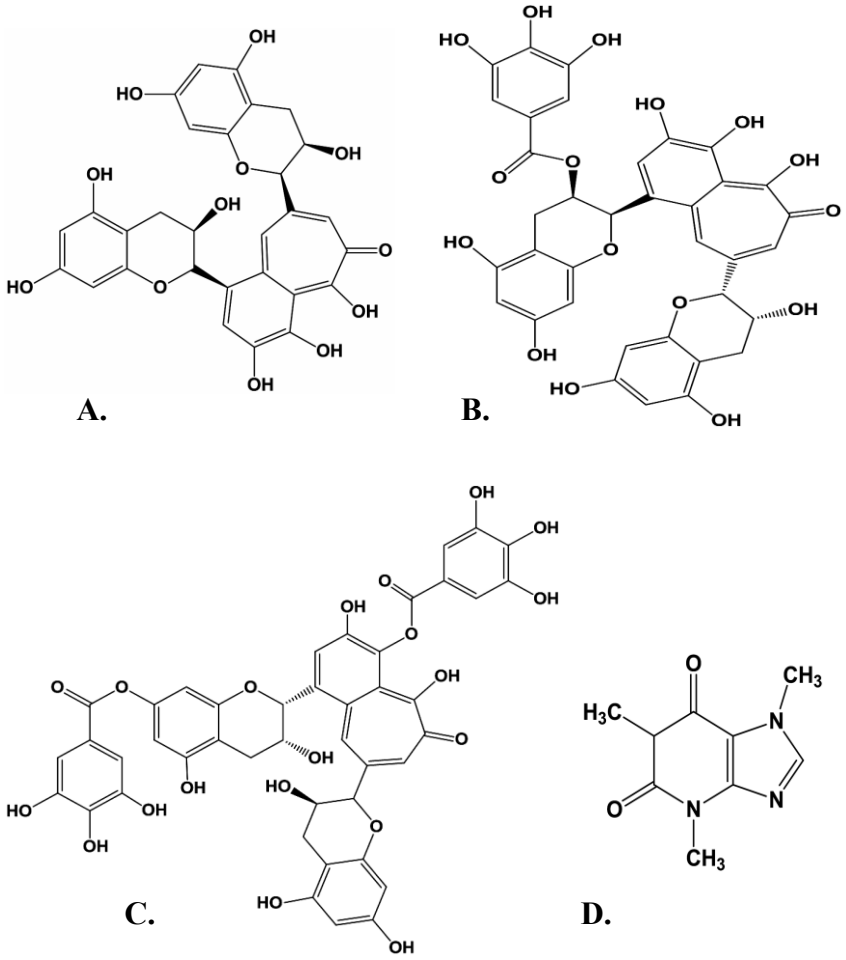


Figure 27. Major theaflavin from black tea (*Camellia sinensis*), having anti-obesity activity probably as AMPK activator, lipolysis inducer, pancreatic lipase inhibitor, and thermogenic agent: **A.** Theaflavin **B.** Theaflavin-3-gallate **C.** Theaflavin-3,3-digallate **D.** Caffeine.

6. *Cyperus rotundus*

The ability of *Cyperus rotundus*, an Indian traditional medicine, to prevent body weight gain in obese Zucker rats was tested. Incorporation of hexane extract of *C. rotundus* tuber into chow diet at either 0.075% or 0.375% dose resulted in significantly decreased body weight gain, and in the end of the study their body weight was lower than control. Food intake and retroperitoneal fat were not significantly changed. No

visible toxicity signs were observed. *In-vitro*, lipolytic activity of the extract was measured in differentiated 3T3-F442A adipocytes expressing ß-adrenergic receptors. Significant increase of fatty acid release was only observed at 250 µg/mL dose (247).

However, there is no further report on the thermogenic activity or other obesity-related activities of this plant in humans. Moreover, the above mentioned study used a hexane fraction. This extract contains a mixture of non-polar compounds such as unsaturated fatty acids which may bind unspecifically to several receptors (248, 249).

7. *Undaria pinnatifida*

Fucoxanthine (Fig. 28) and a glycolipids rich fraction (*Undaria* lipids) from *Undaria pinnatifida*, a popular edible seaweed in Japan and Korea, was incorporated in experimental diet at 0.5% and 2% dose and given to normal Wistar rats fed with normal diet and obese KK-A^y mice fed with high fat diet. In rats with normal diet, no significant difference was found in body weight, food intake, as well as weight of liver and other organs. But the weight of WAT was significantly lower in rats treated with 2% *Undaria* lipids (250).

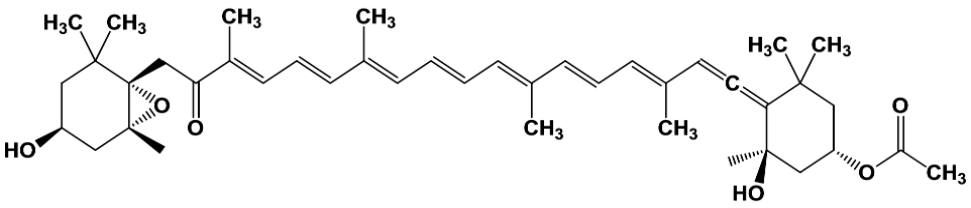


Figure 28. Fucoxanthine from *Undaria pinnatifida*, improves fat metabolism probably via WAT UCP1 activation.

In case of obese mice fed with high-fat diet, 2% extract treatment caused significant decrease in WAT and body weight but not of food intake. When glycolipids and fucoxanthine were given separately, it was shown that fucoxanthine was the responsible active fraction. The weight of BAT was found to be increased in 2% extract treated mice, but there was no significant difference in the BAT UCP1 expression in control and treated groups. Therefore, the decrease in abdominal fat pad weight was not because of an increase of energy expenditure in BAT mitochondria by UCP1. In fact,

UCP1 was present in WAT of 2% *Undaria* lipids and 2% fucoxanthine treated groups. The decrease of WAT weight on rats and mice fed *Undaria* lipids and fucoxanthine diet is thus probably via WAT UCP1 induced fat metabolism (250).

In a sixteen-week, double-blind, randomized, placebo-controlled study, Xanthigen^{TM}, a commercial standardized botanical food supplement containing 300 mg pomegranate seed oil and 2.4 mg fucoxanthin, was found to promote weight loss, reduce body and liver fat content, and improve liver function in obese non-diabetic women (251). In the end of a 16-week trial, resting energy expenditure (REE) of the treated group was significantly higher than the placebo. In this study, the effect of fucoxanthine alone on REE was also observed. Subjects who consumed 4.0 mg and 8.0 mg/day fucoxanthin showed siginificant increase in REE compared to placebo. In another study which used a double-blind crossover study design, the waist circumference and blood pressure of the subjects consuming capsulated seaweed at the dose of 4g/day followed by 6 g/day had decreased (252). There are no further reports conforming the anti-obesity effect of *U. pinnatifida* in humans.

Daily oral administration of 500 and 1000 mg/kg fucoxanthine extract (> 93% purity) for 30 days did not result in any mortality or abnormality in liver, kidney, spleen, and gonadal tissue of experimental mice. However, total cholesterol plasma of the treated mice was significantly higher than control (253). This finding illustrates the need of more detailed studies on the safety of this popular edible seaweed in human objects.

8. *Glycine max*

Glycine max or soya bean is a staple food mainly in Asian countries. It has been suggested that the consumption of soy-based food is correlated to the relatively low occurence of cancer in Japan and China. It is believed that this benefit can be attributed to soya bean isoflavones content (254).

Two main soya protein components; ß-conglycinin (7S-globulin) and glycinin (11S-globulin) were fed to high-fat diet induced obese male normal (ICR) and genetically obese (KK-A^{y}) mice. The proteins were incorporated to an energy restricted diet at 20% dose with casein as control. At the end of the experiment, the body weight of soya protein treated animals was lower than control, with ß-conglycinin having the

most pronounced. Differences in food intake were not significant for all groups. In the ICR ß-conglycinin group, blood levels of triglycerides, glucose, and insulin, were significantly lower than those of casein (255). In another study, 40.6% soya protein isolate was incorporated into calories restricted diet fed to KK-A^y mice. There was no significant difference in body composition of soya and casein control groups. Plasma triglycerides and glucose level were significantly lower in soya group. Adiponectin mRNA expression and its plasma level were increased in soya group, indicating higher fat oxidation activity (256).

Apart from their weak estrogenic activity on the nuclear estrogen receptor, the two main soya isoflavones, daidzein and genistein, can also exhibit biological effects by a non-estrogen receptor mediated mechanisms, such as activation or inactivation of several enzymes as shown in an *in-vivo* study (257). Both daidzein and genistein (Fig. 29) exerted anti-hyperglycemic activity in *db/db* mice, which is thought to be mediated via the activation of glucokinase and inhibition of glucose-6-phosphatase, phosphoenolpyruvate carboxykinase, fatty acid synthase, β-oxidation and carnitine palmitoyltransferase in the liver.

Several human studies regarding the weight reducing effect of soya beans have been reported. It was reported that soya consumers have a lower BMI as compared to non soya consumers. In this study, actual soya consumption was measured by the soya foods frequency questionnaire (258). Obese subjects who consumed a high-soya-protein low-fat diet for 6 months, with or without physical exercise, lost more weight than subjects with a standard diet. They also showed higher BMI and fat mass reduction but there was no reduction of the muscle mass, which is commonly observed in subjects treated with low calorie diets (259). In a 12 week randomized controlled clinical trial, obese subjects treated with soya-based meal replacement formula had a greater weight loss than standard diet group. Five subjects withdrew from the study due to indigestion related adverse effects (260). A larger and longer randomized clinical trial was performed involving obese subjects who were previously diagnosed and being treated for type II diabetes melitus (261). The study period was 12 months. Subjects treated with soya-based meal showed significantly greater weight loss than the standard group. Fasting plasma glucose was significantly reduced in the treated group but only after 6

months treatment. They also showed a greater reduction of sulfonylureas or metformin medication dosages.

Studies on the efficacy of soya proteins to reduce body weight and to increase energy expenditure in obese humans have been reviewed (262). The authors stressed the necessity to conduct long term prospective randomized human trials involving larger number of obese subjects to confirm a long term benefit and safety of soya protein in obese humans.

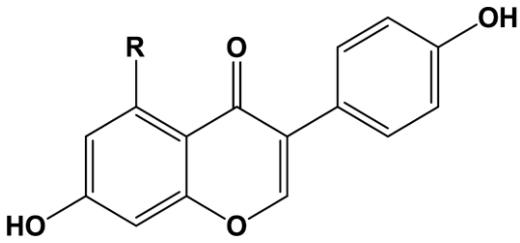
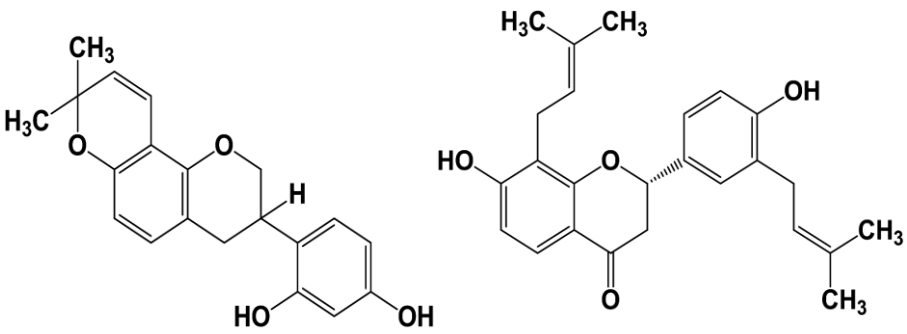


Figure 29. Isoflavones from *Glycine max:* daidzein (R=H), genistein (R=OH).

9. *Glycyrrhiza glabra /G. uralensis*

A licorice hydrophobic flavonoid mixture, prepared by medium chain triglycerides extraction of ethanol licorice root extract, was incorporated in a mice high-fat diet in 0.5%, 1.0%, or 2% dose. Concentration of glabridin (Fig. 30A), a typical licorice flavonoid in this mixture, was 1.2% w/w. Leptin and insulin levels, as well as abdominal adipose tissue mass and size and body weight gain in treated mice were suppressed compared to the control. From DNA microarray analysis, apparently the flavonoids mixture administration has down-regulated ATP citrate lyase and Acetyl-coenzyme A synthetase-2 targeted genes involved in acetyl-CoA synthesis, but up-regulated enoyl-coenzyme A, hydratase/3-hydroxy-acyl coenzyme A dehydrogenase, resulting in suppression of fatty acid synthesis and acceleration of fatty acid metabolism (263).

Another flavonoid isolated form the ethanol extract of licorice, glabrol (Fig. 30B), was reported to inhibit DGAT activity *in-vitro* with an IC_{50} value of 8.0 μM (264).



A. **B.**

Figure 30. Typical flavonoids from *Glycyrrhiza glabra/G. uralensis*, showing anti-obesity effect probably via suppression of fatty acid synthesis and acceleration of fatty acid metabolism: **A**. Glabridin **B.** Glabrol.

Reports on weight reducing effect of licorice in humans are very few. In a placebo-controlled, double blind study, moderately overweight subjects who received licorice flavonoid oil (LFO) at the dose of 300, 600, and 900 mg/day showed a decrease in total body fat content, while significant weight reduction was only found in the 900 mg/day dose (265). The same authors conducted a placebo-controlled, double blind safety study with healthy subjects given a dose of LFO up to 1200 mg/day for four weeks. They found no significant changes in physiology, hematology, or urine of the participants (266).

10. *Capsicum annuum*

Many papers discussed the thermogenic effect of capsaicin (Fig. 5), a pungent compound of red pepper. It was mentioned that consumption of capsaicin-containing food, when the concentration reaches a physiological level, may increase catecholamine secretion in human body (267). Rats intraperitoneally injected by capsaicin (6.0 mg/kg) showed metabolism alterations similar to those in the metabolism of epinephrine intervention, suggesting an activation of β-adrenergic receptor as a mode of action (268). Ingestion of 0.4 g/kg body weight of frozen-uncooked CH-19 Sweet red chili (non-pungent type of red chili) daily during 2 weeks, did not affect the food intake. The

body weight was decreased significantly compared to the control after 3 days treatment, as well as the body fat accumulation especially the visceral area, suggesting the activation of β-adrenoreceptor as the mechanism since the receptor is found to be higher in that part. Capsiate, the main capsinoid in CH-19 Sweet which structure is similar to capsaicin was thought to be the active compound (Fig. 31) (269), since the administration of 10 mg/kg body weight capsiate via stomach tube daily in mice was found to increase the expression of *ucp1* mRNA in BAT, *ucp2* mRNA in WAT, and *ucp3* in muscle (270). The study regarding the safety and efficacy of capsinoids in humans has been recently reported (271). Oral administration of 6 mg/day capsinoids oil extracted from CH-19 Sweet was found to be safe and associated with an increase in fat oxidation. Of 13 genetic variants tested, *TRPV1* (transient receptor potential cation channel, subfamily V, member1; vanilloid receptor 1 (VR1) or capsaicin receptor), Val585Ile and *UCP2* –866 G/A were found to correlate with abdominal adiposity change.

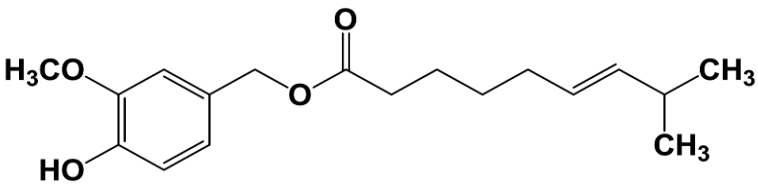


Figure 31. Capsiate, unpungent capsinoid from CH-19 Sweet red chilli (*Capsicum annuum*) which shows anti-obesity activity by increasing fat oxidation.

11. *Rubus idaeus*

Raspberry ketone (4-(4-hydroxyphenyl) butan-2-one (RK) (Fig. 32) is a major compound in the red raspberry (*Rubus idaeus*) fruit. Rats fed with high-fat-diet plus RK (particularly at 2% dose of the diet) had a significantly lower final body weight, as well as liver and visceral adipose tissues. Raspberry keton alone did not stimulate lipolysis or bind to β-adrenergic receptors but it acted synergistically with norepinephrine to induce lipolysis at the concentration of 10^{-3} M. At the same concentration it did not enhance the HSL activity but the amount of HSL protein in the fat layer was increased. Thus, the

proposed mechanism is by increasing the translocation of HSL from cytosol to the lipid droplets (272). There are no further reports on the efficacy of this plant either in animals or humans.

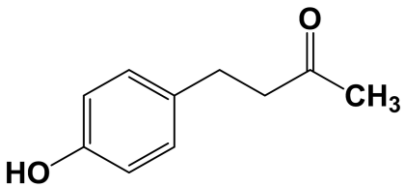


Figure 32. Raspberry ketone (4-(4-hydroxyphenyl) butan-2-one from *Rubus idaeus*, showing synergistic effect with norepinephrine to induce lipolysis.

12. *Evodia rutaecarpa*

Evodiamine is a major alkaloid in Evodia fruit (*Evodia rutaecarpa,* Rutaceae) (Fig. 33). *Evodia* fruit ethanol extract was incorporated into a high fat diet at dose 1.35% (contains 0.02% evodiamine). After 21 days of treatment, the evodiamine treated rats had a lower final body weight, smaller perirenal and epididymal fat than those of the control group. The evodiamine group also has a better lipids and sugar plasma profile, as well as lipids liver profile. Based on the two liver damage indexes, glutamic-pyruvic transaminase (GPT) and glutamic-oxalacetic transaminase (GOT), no liver toxicity was observed. Subcutaneous administration of evodiamine at 1-3 mg/kg body weight dose resulted in a hypothermic effect in the food fasted mice, but in the food satiated mice the higher dose of 10 mg/kg body weight was needed to induce the effect. This hypothermic effect was inhibited by pretreatment with capsazepine, a vanilloid receptor antagonist. Specific mitochondrial guanosine diphosphate (GDP) binding in BAT mitochondria was also found to be higher in the treated group (273).

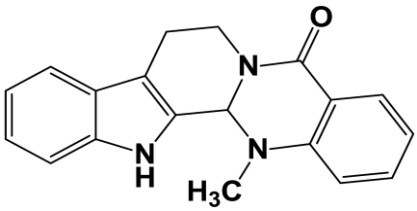


Figure 33. Evodiamine, major alkaloid in *Evodia rutaecarpa*, showing hypothermic effect especially in the food fasted mice.

In a double-blind, randomized and placebo-controlled clinical trial, obese women who received 3 g *Evodia* extract (containing 6.75 mg evodiamine and 0.66 mg rutaecarpine) did not show significant reduction of body weight or increment of resting metabolic rate as compared to placebo group. Several adverse effects such as headache, insomnia, dizziness, and constipation were reported for the Evodia group although not siginificant (274). Despite the lack of any further efficacy study in humans, this fruit is used as an ingredient of commercial slimming preparations.

13. *Nelumbo nucifera*

Rats fed by a high-fat diet contains 1% (w/w) *Nelumbo* ethanol extract have a significantly lower final body weight compared to the control group but the food intake was not affected. Several flavonoids have been isolated from this plant, those are quercetin 3-*O*-α-arabinopyranosyl-(1→2)-β-galactopyranoside, hyperoside and isoquercitrin, (+)-catechin, and astragalin (Fig. 34).

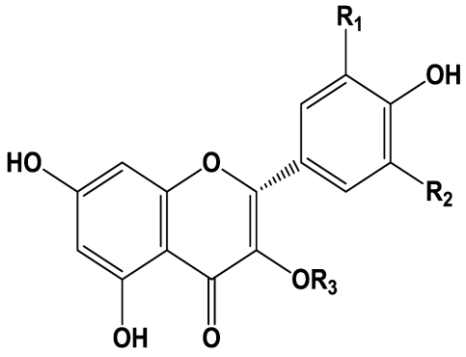


Figure 34. Flavonoids from *Nelumbo nucifera*, showing thermogenic and lipolytic activity probably via activation of UCP3 and β-adrenoceptor: Quercetin 3-*O*-α-arabinopyranosyl-(1→2)-β-galactopyranoside (R1= H, R2= OH, R3= α-arabinopyranosyl-(1→2)-β-galactopyranoside), hyperoside (R1= OH, R2= H, R3= galactose), isoquercitrin (R1= OH, R2= H, R3 = glucose), (+)-catechin (R1= H, R2= OH, R3= H), astragalin (R1=H, R2= H, R3= glucose).

They showed lipolytic activity in 100 μM concentration. This lipolytic activity was abolished by the addition of Propanolol 10 μM, a β-adrenoceptor antagonist, suggesting activation of β-adrenoceptor as the mode of action (275). Additionally, as previously mentioned in this review, this plant extract also showed the upregulation of UCP3 in a rat muscle, suggesting its potential as a thermogenic agent (121). However, there is no report on the efficacy of thermogenic acitivity of this plant in humans.

14. *Solanum tuberosum*

The ethanol extract of a new potato variety containing purple pigments, *Solanum tuberosum* L. cv. Bora Valley, was tested for the proliferation and differentiation of 3T3-L1 preadipocytes as well as the leptin and insulin levels. It was found that the extract significantly inhibited the proliferation and differentiation of 3T3-L1 preadipocytes at 10 μg/mL concentration, reduced the leptin and insulin levels at 500 mg/kg in high fat diet fed rats. Oral administration of the extract at the dose of 200 mg/kg body weight for 4 weeks did not reduce rats' body weight although hyperlipidemic parameters and the size of abdominal fat were significantly improved. The expression of p38 MAPK in 3T3-L1 adipocytes was downregulated but the expression of ERK was unchanged, whereas the *ucp-3* mRNA expression in the fats and liver tissues of high fat diet fed rats was upregulated. Thus, the authors suggest that the anti-obesity of the extract is exerted via inhibition of lipid metabolism and induction of thermogenesis through p38 MAPK and UCP-3 pathways subsequently. Anthocyanins are thought to be the active compounds but this needs to be confirmed by further studies (276).

15. *Coffea sp*.

Coffee is one of the most widely consumed beverages in the world. The stimulant effect of coffee is attributed to its main alkaloid, caffeine (Fig. 27D). Several studies reported the thermogenic effect of caffeine in humans. Caffeine was found to dose-dependently increase energy expenditure in healthy subjects with moderate caffeine habitual intake, as compared to placebo (277). The caffeine was orally administered at the dose of 100, 200, and 400 mg/day. In another study, either caffeine (8 mg/kg body weight) or caffeinated coffee (4 mg/kg body weight caffeine) increased

the metabolic rate in normal and obese subjects. The authors also studied the effect of the caffeinated coffee after a meal. They found that the thermic effect of the food after caffeinated coffee was greater than after decaffeinated one. The fat oxidation was also increased in the caffeinated coffee group (278). It was reported that consumption of 100 mg caffeine/day increases metabolic rate and 24 h energy expenditure of lean and post-obese subjects (279). A prospective study reported that there is a correlation between an increased coffee and tea consumption with less weight gain (280). However, more trials on the ability of coffee or caffeine to cause weight loss in humans are needed. Besides, it is also possible that there are other compounds other than caffeine presence in coffee could contribute to the thermogenic effect of coffee.

Table 1. Summary of botanicals used as anti-obesity herbs and their mechanisms (+ = tested and active/potential for clinica trial, +/– = potential for clinical trial but needs more data, - = tested but inactive/not potential for clinical trial, nm = not mentioned).

Plant's name	Reduce body weight	Active compound (s)	Mechanism(s)									Potemtial for clinical trial	Reported active dose/duration of use in humans, references	Remarks
			Central appetite suppressant	Delaying gastric emptying	Amylase inhibitor	Lipase inhibitor	PPAR γ	PPAR α	AMPK	UCP	B-Adrenoceptor			
Hoodia sp.	+	3β-[β-D-thevetopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranosyloxy]-12β-tigloyloxy-14β-hydroxypregn-5-en-20-one	+	nm	nm	nm	nm	nm	nm	nm	nm	+/–	_	No further data on efficacy and toxicity in humans.
Benincasa hispida (Thunb.) Cogn	nm	nm	+	nm	nm	nm	nm	nm	nm	nm	nm	_	_	Lack data both *in-vitro* and *in-vivo* efficacy.
Mitragyna speciosa Korth.	+	Mitragynine	+	nm	nm	nm	nm	nm	nm	nm	+	_	_	Addictive side effect.
Carraluma fimbriata Wall.	+	nm	+	nm	nm	nm	nm	nm	nm	nm	nm	+/–	0.5 - 1 g/60 d (84)	Need more data on efficacy and safety in humans.

Catha edulis Forsk	nm	Cathinone	+	+	nm	nm	nm	nm	nm	nm	+	–	–	Side effects similar to amphetamine.
Capsicum annuum L.	+	Capsaicin, capsiate	+	nm	nm	nm	nm	nm	nm	+	+	+/–	6 mg capsinoids oil/2 wk (270)	Need more data on efficacy and safety in humans.
Garcinia cambogia Desr.	+	(⁻)-hydroxycitric acid	+	nm	nm	nm	nm	nm	nm	nm	nm	–	–	No efficacy found in humans.
Cyamopsis tetragonolobus L.	+	Galactomannan	–	+	nm	nm	nm	nm	nm	nm	nm	–	–	Many contradictory reports on efficacy in humans.
Amorphophallus konjac K. Koch.	-	Glucomannan	–	nm	nm	nm	nm	nm	nm	nm	nm	–	–	Many contradictory reports on efficacy in human.
Panax ginseng C.A. Meyer	+	Ginsenosides Rg1, Re, Rg2, Rb1, Rc,Rb2, Rb3 and Rd	+	nm	nm	+	+	nm	+	+	nm	+/–	100 – 150 mg standardized extract/8 – 9 wk, twice a day (281)	Need more data on efficacy and safety in humans.
Panax quinquefolius L.	–	Ginsenosides Rg1, Re, Rg2, Rb1, Rc,Rb2, Rb3 and Rd	nm	nm	nm	+	nm	nm	nm	+	nm	+/–	3 – 9 g/duration time not mentioned (146)	Need more data on efficacy and safety in humans.
Lagerstroemia speciosa (L.) Pers.	+	Corosolic acid, lagerstroemin, flosin B, reginin A	nm	nm	+	nm	+	+	nm	nm	nm	+/–	32 – 48 mg / 2 wk (119)	Need more data on efficacy and safety in humans.
Coix lachrymal-jobi L.	+	nm	nm	nm	nm	nm	nm	nm	nm	nm	nm	–	–	Lack data on efficacy in humans.

Hibiscus sabdariffa L.	+	(+)- allo-hydroxycitric acid	nm	nm	+	nm	nm	nm	nm	nm	nm	–	–	Lack data on efficacy in humans.
Nelumbo nucifera Gaertn.	+	nm	nm	nm	+	+	nm	nm	nm	+	+	–	–	Low *in-vitro* activity.
Phaseolus vulgaris L.	+	nm	nm	nm	+	nm	nm	nm	nm	nm	nm	+/–	445 mg /30 d (123)	Many contradictory reports on efficacy in humans.
Triticum aestivum L.	+	nm	–	nm	+	nm	nm	nm	nm	nm	nm	+/–	nm (130)	Need more data on efficacy and safety in humans.
Morus alba L.	+	1-deoxynojirimycin	nm	nm	+	nm	+	+	nm	nm	nm	–	–	Lack data on efficacy in humans. Low *in-vitro* activity.
Aframomum meleguetta (Roscue) K. Schum.	nm	nm	nm	nm	nm	+	nm	nm	nm	nm	nm	–	–	Lack data on efficacy in humans. Low *in-vitro* activity.
Spilanthes acmella (L.) Murray	nm	nm	nm	nm	nm	+	nm	nm	nm	nm	nm	–	–	Lack data on efficacy in humans. Low *in-vitro* activity.

Salix matsudana Koidz.	+	Apigenin-7-*O*-D-glucoside, luteolin-7-*O*-D-glucoside, chrysoeriol-7-*O*-D -glucoside	nm	nm	+	+	nm	nm	nm	nm	+	–	–	Lack data on efficacy in humans. Low *in-vitro* activity.
Glycyrrhiza glabra L.	+	Licochalcone A	nm	nm	+	nm	nm	nm	+	nm	nm	–	–	Effective dose in human is not reasonable, 900 mg licorice flavonoids oil/day (265).
Punica granatum L.	+	Tannic acid, ellagic acid	nm	nm	nm	+	nm	nm	nm	nm	nm	+/–	–	Lack data on efficacy in humans.
Rosa canina	+	*Trans*-tiliroside	nm	nm	nm	nm	nm	+	nm	nm	nm	–	–	Lack data on efficacy in humans.
Pinus densiflora SIEB. *et* ZUCC	+	nm	nm	nm	nm	nm	+	nm	nm	nm	nm	–	–	Lack data on efficacy in humans.
Camellia sinsensis (L.) O. Kuntze	+	Epigallocatechin gallate, theaflavins, caffeine	nm	nm	nm	+	nm	nm	+	nm	+	+/–	270 mg – 1200 mg green tea extract (241), or green tea extract equal to 50 mg caffeine and 90 mg EGCG (166)	Need more data on efficacy and safety in humans.
Zingiber mioga Ros.	+	nm	–	nm	nm	nm	nm	nm	nm	nm	nm	–	–	Lack data on efficacy in humans.

Zingiber officinale	+	6-shogaol, 6-gingerol	–	nm	nm	+	+	nm	nm	nm	nm	–	–	Lack data on efficacy in humans.
Vitis vinifera Linn.	+	Resveratrol, vitisin A	nm	nm	nm	nm	+	nm	+	+	nm	–	–	300 mg grape seed extract/day did not give significant difference in 24 h thermogenesis compared to placebo (218).
Citrus sp.	+	Cyanidin, naringin, naringenin, isonaringin, narirutin, hesperidin,synephrine,octopamine	nm	nm	nm	nm	nm	nm	nm	nm	+	+/–	350 mg extract of *Citrus sp* mixture/12 wk (222), *C. aurantium* extract (26 mg synephrine, 4 mg octopamine (227).	Need more data on efficacy and safety in humans.
Salvia miltiorrhiza Bunge	+	Cryptotanshinone	nm	nm	nm	nm	nm	nm	+	+	nm	–	–	Weak AMPK activator and DGAT inhibitor *in-vitro*, no report on efficacy in humans.
Cyperus rotundus L.	+	nm	nm	nm	nm	nm	nm	nm	nm	nm	+	–	–	Possible false positive. No efficacy report in humans.

Undaria pinnatifida (Harvey) Suringar	–	Fucoxanthine	nm	nm	nm	nm	nm	nm	nm	nm	nm	+/–	4 mg fucoxanthine, 16 weeks (251)	Possible side effect: Mice fed with 500 mg fucoxanthine/day for 30 days showed an increase of total cholesterol plasma (253). Need more data on efficacy and safety in humans.
Glycine max (L.) Merr.	+	Daidzein, genistein	nm	nm	nm	nm	nm	nm	nm	nm	nm	+/–	1 – 5 portions of high soy protein diet, 3 – 12 months (261, 282)	Need more data on efficacy and safety in humans.
Rubus idaeus L.	+	4-(4-hydroxyphenyl) butan-2-one	nm	nm	nm	nm	nm	nm	nm	nm	nm	–	–	No report on efficacy in humans.
Evodia rutaecarpa (Juss.) Benth	+	Evodiamine	nm	nm	nm	nm	nm	nm	nm	nm	nm	–	–	No significant effects in subjects receiving 3 g Evodia extract/day for 8 weeks (274).
Solanum tuberosum L.	–	nm	nm	nm	nm	nm	nm	nm	+	+	nm	–	–	No report on efficacy in humans.
Coffea sp.	+	Caffeine	nm	nm	nm	nm	nm	nm	nm	nm	+	+/–	4-8 mg caffeine/kg body weight (277, 278)	Need more data on efficacy and safety in humans.

Conclusion

Obesity results from the imbalance between energy intake and energy expenditure. Therefore, obesity treatment is focused on reducing energy intake (by suppressing the appetite and delaying or inhibition of nutrition absorption) and increasing energy expenditure (by blocking adipogenesis or inducing lipolysis followed by fat oxidation). As described in this review, many plant screening projects have been done, including all important targets. Some plants have been reported to be active in more than one pathway or mechanism, such as *Mitragyna speciosa, Catha edulis, Lagerstroemia speciosa*, and *Panax ginseng*. The summary of mechanisms involved in anti-obesity activity of herbs reported in this review is presented in Table 1. Some active constituents are present or can be incorporated into food for daily consumption, such as capsaicin, resveratrol, and isoflavones. A few candidates went into clinical trials, such as P57 from *Hoodia gordonii,* but none have reached the final stage for registration.

However, despite of insufficient data for safety and efficacy, many are available as nonprescription herbal preparations. Examples are *Citrus aurantium* containing synephrin, which is the hope after the *Ephedra* ban; hydroxycitric acid from *Garcinia cambodia* which reduces appetite and induces thermogenesis; or the amylase inhibitors from *Phaseolus vulgaris*. Extensive research has been done on several candidates such as green tea, black tea, ginseng, and soya. Mechanism and responsible compounds have been elucidated but whether they will go further into clinical trials remains unclear. The major question being asked is whether the effect is at a reasonable dose. Exploration of traditionally used plants could be very beneficial to uncover more potent candidates, such as the P57 experience, a good balance between data supported by scientific work and advertisement is needed to avoid dissatisfaction. The recently expanded metabolomics approach along with multivariate data analysis could be a valuable tool in obesity herbal discovery since it offers the potential of studying efficacy and safety in a holistic approach and thus would also reveal the presence of pro-drugs or synergy. It is also important for the quality control of herbal medicines.